ADRENAL CORTEX

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JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

FRANK FREMONT SMITH
Medical Director

With the accelerating rate at which new knowledge is accumulating and with the increasing recognition that nature is of one piece it becomes evident that the continued isolation of the several branches of science from one another is a erious obstacle to scientific progre s

Nowhere in science is the need for combined operations more evident than in medicine Today to be effective medical research and practice must embrace data from all the disciplines including nuclear physics at one end of the pectrum and cultural anthropology at the other for advances in one field are frequently dependent upon knowledge derived from quite another discipline

Although the fertility of the multi-discipline approach is thus recognized universities scientific societies and journals have not yet made adequate provision for channels of interdisciplinary communicatio

The Josiah Macy Jr Foundation therefore has endeavored to meet this need by bringing together for a series of two day annual conferences a small group of investigators representing in so far as possible all the branches of cience which bear on a chosen problem These round table discussions of research experience concepts and plans are conducted in a friendly and informal atmosphere which promotes communication cross fertilization of ideas and cooperation The success of such an endeavor is dependent upon full participation of all members in the di cus ion Accordingly the attendance at any conference is limited to twenty five

Under the guidance of Dr Willard C Rappleye President of the Foundation since 1942 the Conference Program has been gradually expanded and enlarged until it now includes thirteen different groups which meet annually to discuss a wide variety of problems in the field of medicine and the closely related disciplines The Conference Program has become a major interest of the Foundation

In order to share with a wider group of investigators and students the essential quality of these conferences the informal nature and tempo of the discussions in so far as possible are preserved in the published transactions

INTRODUCTION

C N H LONG,
Chairman

This conference is particularly pertinent in view of the great interest that has developed in the use of the adrenal steroids in clinical medicine, and the scarcity of the substances that are active in the relief of rheumatoid arthritis and other conditions In addition, intensive search is going on for other sources of starting material so that an adequate supply of cortisone and related substances may be made available

In view of the very large number of steroids that have been isolated from the adrenal, many apparently without known biological activity I thought it would be appropriate to ask Dr Kendall, who has contributed so much to this particular topic to open our discussion

RELATION OF CHEMICAL STRUCTURE OF ADRENAL CORTICAL HORMONES TO BIOLOGICAL ACTIVITY

EDWARD C KENDALL
Department of Physiological Chemistry
The Graduate School University of Minnesota The Mayo Foundation

YOU ARE all familiar with the formulas in Figure 1 Those are the structures of the hormones which have been regarded as the ones furnishing the physiological activity of the secretion of the adrenal cortex They have been subjected to many criteria for their activity In regard to the effect on carbohydrate metabolism and deposition of glycogen in the liver, it has been shown that there are differences but they are quantitative not qualitative except that desoxycorticosterone does not deposit glycogen nor does the amorphous fraction appreciably However there is no more than perhaps a two fold difference between the others

In regard to atrophy of the adrenal gland moderate doses of compounds A, B E and F will produce greater atrophy than will desoxycorticosterone although large amounts of desoxycortico sterone will produce atrophy of the adrenal and thymus

There is not a great deal of difference in the cold test as developed by Dr Selye and others and in Dr Ingle s test with the response to muscle I believe compound F is about as active as compound E

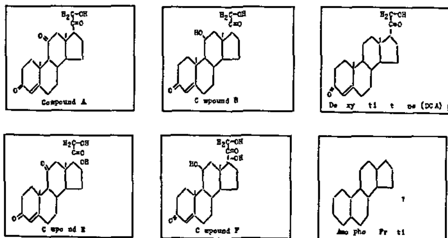


FIGURE 1

Ingle Not according to my own data Mr Pabst published data which indicated that "F" is about 92 percent more active than 'E", but my own results indicate that 30 percent is more nearly correct

Kendall With the muscle test?

Ingle Yes

Kendall We are then confronted with this surprisingly large number of hormones in the adrenal and the possibility that there would have to be a certain amount of each one to make a balanced secretion This is a rather complicated situation However, these tests were done on small animals since there was not enough to try them in the human being Then again there are different amounts of the hormones present in the glands of two types of animals the beef and the hog

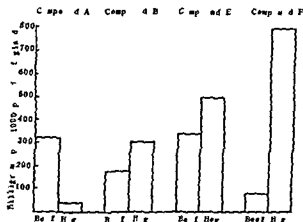


FIGURE 2

Figure 2 shows the amounts of compounds A, B E and F in the adrenals of the beef and the hog You see in the beef there is a *large amount of A 300 mg in a thousand pounds of beef glands* and very little in the hog more of B in the hog than in the beef much more of E than F in the beef, and a very large amount of F in the hog This raised the question of what is in the human adrenal gland These results were carried over more or less bodily in the interpretation of the secretion in the human The first suggestion that there was a difference between the human and the lower animals came with the use of the acetate of compound A When this became available in 1946 it was given to a patient with Addison's disease and we were surprised to find that even at a level of 200 mg a

day it had almost no effect The first striking difference between the compounds E and A in the human came when we tried it on patients with rheumatoid arthritis One hundred milligrams of compound E will relieve the symptoms of rheumatoid arthritis, restore a normal sedimentation rate and produce a sense of well being With A the result is simply a blank Nothing happens at all That was quit surprising since A had been shown to be active in the lower animals With respect to rheumatoid arthritis, with desoxycorticosterone there may be an increase in the symptoms Compound F was about as active as E but not more so That has made me feel that in the human, compounds A and B are simply precursors of E and F and are not delivered from the gland in the secretion but are probably retained and further elaborated into E or F which are the active hormones Dr John Schneider at Jefferson Medical College in Philadelphia has isolated compound E 50 mg from 1,000 liters of normal urine Dr Mason has not isolated compound E from normal urine but he has isolated a surprisingly large amount of compound F from patients with tumors of the adrenal but neither of these investigators has seen any traces of A or B So perhaps the normal secretion contains only E or F, although we cannot dismiss the presence of the amorphous fraction which controls renal function so largely At any rate that simplifies the probable nature of the secretion from the adrenal We do not have to balance up four compounds

The present interest in the structure of the hormones is so great because of the urgency to find something which will act just as well and in place of compound E in clinical medicine

The greatest interest centered around Reichstein s compound S Dr Gallagher made some, I believe chemists at Parke Davis and Co made some and Dr Percy Julian of the Glidden Company made 600 gm There was enough to send around and we received some samples of Reichstein s compound S and gave it to some patients These patients also received both cortisone and ACTH before or after they received compound S We gave one patient 100 mg of compound S a day then 200 and finally 250 mg without the slightest change in her condition or sedimentation rate She was then treated with compound E and responded beautifully She was by the way perhaps the most severe case of any that we have treated She could not turn over in bed Every joint was swollen and hot and she ran a temperature every day She was in miserable shape but in a few days with compound E these all subsided After a few days more she was up and around The severity of her condition was so great that perhaps it was asking too much

to have a demonstration of a compound about which we knew nothing Certainly compound S did not produce any change We then treated another patient who had received both E and ACTH He was given 200 mg a day for a week At the end of that time he was asked how he would estimate the change compared with the change he had secured with cortisone and he said, 'Perhaps 15 percent' but he was not very sure of even that

Thorn What was the effective dose of cortisone in that particular patient?

Kendall One hundred milligrams The third case was a patient who had been treated with cortisone with a very favorable response and she responded not at all less than 10 percent She received 200 mg of compound S a day and I believe at the end she received 300 So we feel that there is very little to be expected from compound S The only difference of course, is the atom of oxygen on carbon 11 Had compound S been active, it would have changed the situation Instead of looking and looking for ways to make cortisone we would simply produce compound S in large amounts

I should like to mention some other compounds 4,5 Dihydro F is not active It has everything, exactly the same as compound E, the OH at 17 and the oxygen at 11, but without a double bond it does not produce any effect whatsoever We have used the triol 17, 20, 21 with the ketone at C 20 reduced to a hydroxyl group That is without value although interestingly enough with the triol one patient did have a drop in the sedimentation rate Another interesting point I should like to mention is that when Upjohn's total extract of the adrenal was given to another patient the sedimentation rate went down to one mm per hour and yet the condition of the patient did not parallel that change at all He was not improved more than about 50 percent perhaps and yet his sedimentation rate was certainly normal

Selye What was the dose of that?

Kendall Very high

Selye That is a lipo extract?

Kendall Yes, given by mouth I was informed it was about $340 worth a day

I have some new work which has not been published In the partial synthesis of cortisone we found an impurity which after some effort was identified as 6 dehydrocortisone If you are very careful and exclude air in the presence of hydrogen bromide the percentage of this can be as low as 0 20 or so It is very fortunate that the absorption spectrum of this diene ketone is so high 27 000 at 280 μ so by taking a fairly large sample you can measure

down to 1/10 of one percent If you are not careful this impurity can be as high as 4 5 percent

Well the question was is this a toxic substance? Does it antagonize cortisone or is there a synergistic action? In other words, what must be done in the manufacture of cortisone? Can it be neglected or must it be removed? We did not take any chances when we discovered it and sought ways to remove it Fortunately it can be removed very easily You merely have to shake the compound in solution with zinc dust this bond is reduced out and the compound is changed back to cortisone Curiously enough if there is a mixture of the two you cannot remove the diene by crystal lization It separates with compound E As a matter of fact the concentration changes very little It is neither concentrated in the crystals nor in the mother liquor We tried many solvents many conditions It cannot be separated conveniently in any way Chroma tography does not separate it and when we wanted to make the diene free from cortisone to see its effect we ran into a great deal of difficulty in its preparation The preparation of the diene has taken about a year s time We now have it in pure form and I was quite interested to have it tested to see whether it resembled cortisone or what its effect was bearing in mind that every compound that differed from E failed to produce the physiologic effect of com pound E I should add that when the diene is mixed with compound E it does not depress the melting point so it certainly is a very peculiar compound in its chemical and physical properties

Dr George Higgins tried it out first on normal rats, and on adren alectomized rats I am indebted to Dr McGinty of Parke Davis and Co for tests in regard to the deposition of glycogen The normal rats weights went down with 2 mg of cortisone that is, 2 mg a day The weights of the normal rats with the diene went up The weights when the two compounds were mixed together 2 mg of each a day went down still more so there is a synergistic action between the two

In regard to the deposition of glycogen there was not much to choose between the two compounds It deposits glycogen almost the same as compound E In the adrenalectomized animal we found this same relationship The adrenalectomized animals with the diene did rather well they did not lose weight the fur was slick and they looked normal With compound E they obviously were not well The diet was such that the uncontrolled untreated adren alectomized animal did not survive more than six or seven days Two out of 10 did not die in 10 days but accessory adrenal glands were found in them The surprising result was in the effect on the

blood cells In the normal animal cortisone suppressed the leuko cytes The normal value is about 16,000 cells This went down to 6 000 with cortisone, and the diene instead of suppressing raised the white count to 18,000 in 11 days

Long This is the total white count, Dr Kendall?

Kendall Yes, the total

Loeb Is this in the adrenalectomised animal?

Kendall No normal In the adrenalectomized rat the effect was not so evident but there is a qualitative difference of these two compounds which differ only by a double bond one from the other

Some few days ago the director of the Department of Pharma cology of a large manufacturing firm was in Rochester and he asked me if I would not tell him a simple test so that they could screen compounds and tell which ones would be good for the treatment of rheumatoid arthritis My answer was that the problem is very simple All he had to do was to examine the compound If it was cortisone it would work If it was not cortisone it would not "Well', he said, "I won't take that I am going home to find out

'It will cost you thousands of dollars'

'Maybe it will," he said

However, I don t think he is going to find one very easily

This highly specific effect of structure was unforeseen and raises the question of the influence of these various steroids on the compo sition of the cells in the blood, and their distribution I do not believe that compound A will produce this effect

Ingle I don't know

White Compound A will lower the lymphocytes

Kendall Strikingly?

White Significantly, at least in rats and mice

Kendall E does of course

White Yes

Kendall In respect to atrophy the diene will atrophy the adrenal gland more than does cortisone but it will not atrophy the thymus so much Incidentally the adrenal of the guinea pig is not atrophied at all with cortisone I don t know whether that is in the literature

Pincus Does this diene do anything to the blood count of the adrenalectomized animal?

Kendall Yes With the diene the count was not nearly as high as in the normal rat but it was higher than with cortisone

Sayers Does the diene cause atrophy of the adrenal gland of the guinea pig?

Kendall I don t know

DISCUSSION

Long Dr Kendall, I don't know whether you would care to comment in passing on a very practical matter which you referred to We have all read at least in the newspaper of the various possible sources of starting material for the synthesis of cortisone Do you care to say what actually is the real situation? Is the Mexican yam to be looked forward to? Is sarmentogenin, the material from the strophantus sarmentosus actually a C 11 compound?

Kendall Yes it is

Long There seems to be a difference of opinion I read a French review in which the oxygen was placed at 12

Kendall It is at 11 I have been informed that Reichstein has recently received 12 samples of strophantus sarmentosus He examined those for sarmentogenin From those 12 only one contained any sarmentogenin

Long I have his reprint on that

Kendall There are two points about that plant which make it rather hopeless One is that it does not bear seed until it is five years old and the other is that the seeds drop out very soon after the pods open The seeds do not contain sarmentogenin until they are ripe, so that you have to gather them very promptly You could not do that with an enormous crop so I would say it was rather hopeless

Kendall These pods come out from the stem and just the minute they open the wind takes the seed out from the mechanical standpoint that looks hopeless

Pincus Use plant growth hormone to have them come in!

Kendall Will they ripen?

Pincus Yes

Long What about the Mexican yam Dr Marker s material?

Kendall The position of the oxygen is in doubt I attended a meeting in Chicago three weeks ago today at which new work was reported In breaking down the steroid you should get the 3, 12 diketo of either the cis or trans structure of the A and B rings The product so obtained did not correspond to either There is a ketone group but the position is in doubt In the November 14th issue of Chemical and Engineering News there is an article by Marker with all the various possibilities (Marker Russell E and Applezweig Norman Steroidal Sapogenins as a source for cortical steroids *Chemical Engineering News 27 348 (1949)*) I think the uncertainty is how much is available I don t know that he has given that I suppose it varies at different times of the year but it seems to me that a lot of time will have to be spent in the exploration of

which is the best So it will take I would say two or three or four or five years, but I think you would like to hear about a thing which gave me a great thrill, and that is the total synthesis I would not be a bit surprised if the organic chemists are going to give you the first answer I certainly believe it will be done in four or five years It may be done in one year Very substantial progress has been made

Long I don't want to anticipate what Dr Bloch will have to say to us but I wonder if in view of the importance of the problem sufficient attention has been paid to the possibilities of the enzymatic transformation After all, the adrenal gland does carry out with great expediency this total synthesis from the C 2 fragments Do you know of anybody making an attempt to study the enzymatic systems of the gland that are capable of producing quite a variety of C 11 steroids at least according to isolation techniques?

Kendall If you will bear in mind the amount of cortisone required it will come outside of the boundaries of any enzymatic activity unless you grow the enzyme

There is another thing you would like to know We have treated 20 25 cases of rheumatoid arthritis Only one or two patients required more than 100 mg a day One patient, a large man weighed over 200 pounds the sedimentation rate did not come down to normal, he was not fully relieved so we gave him 150 mg a day He responded to the last 50 mg and apparently required it However in most cases that is not true

Recently we have been treating other conditions One was a patient with dermatomyositis ten years old, we gave her 100 mg a day I don't think she weighed more than 50 60 pounds, so that it was a very large dose but she did not show any signs whatever of overdosage Apparently it was being destroyed At least she metabolized it so she was not suffering from an overdosage She did respond very well There were also two cases of lupus erythematosis who required more than 100 mg Those figures which I gave you are minimal With chronic ulcerative colitis, two cases responded and the last case with 100 mg did not respond at all I am certain if this patient had received 300 maybe 400 mg for a few days there would have been a response and I don't think he would have been overdosed When ACTH is given of course you never know how much cortisone is released When you get amounts of cortical steroids in the urine as high as 17 mg and you get only 2 or 3 mg from 100 mg of cortisone it must be a very large weight that is liberated by ACTH

Conn We have just finished a comparative study in a normal individual who was given ACTH on three separate occasions for

prolonged metabolic studies The initial study was with 50 mg of ACTH a day for a period of ten days and then subsequently, after a period of several months of rest 68 mg a day, and then later 100 mg a day For comparative purposes a fourth study was done on this same individual this time using 200 mg a day of cortisone The metabolic responses with 200 mg of cortisone per day are about 50 percent as intense as those observed with 50 mg a day of ACTH

Long That coincides with your figures, Dr Kendall, as 2 against 1?

Kendall Yes The only point there is unless it was the same sample of ACTH it would be hard to know that you had exactly the same activity per milligram

Conn The comparative ACTH responses were progressively more intense with increasing doses

Thorn May I ask Dr Kendall about one point? In your adrenal ectomized rats what dose of compound E maintains a normal growth curve? With 2 mg of E you must have been overdosing the animals since you got loss of weight Would you not want to compare your extra double bond compound with the amount of cortisone necessary to maintain weight?

Kendall In the adrenalectomized animal?

Thorn Yes the adrenalectomized animal

Kendall Yes certainly but we wanted to get the answer We picked those figures out of the sky

Thorn It would appear essential to determine what dose of cortisone if any maintained a normal growth curve in adrenalecto mized animals It would then be possible to interpret comparative effects of an unknown steroid such as the diene you have mentioned

Kendall I think there will be different figures if we repeat that experiment with smaller doses On the other hand you get into trouble if you decrease the amount of compound E sodium chloride is lost

Thorn That is my question Can you maintain normal growth in a rat with E?

Kendall I don't think we can

Thorn Why do you say that E is the compound? I thought that your summary from your four formulas was that E was the com pound that the adrenal was most likely to be releasing

Kendall In addition I said that you must postulate the amor phous fraction which has its greatest activity on renal function You cannot reduce the number of hormones to any less than those two

Ingle How old were the rats in which you studied growth? Were they immature rats?

Kendall No

Ingle There is an age difference in response to cortisone We have never been able to sustain growth in adrenalectomized male rats of 200 grams with any dose of cortisone, yet adrenalectomized newly weaned rats are able to continue growth, although at a sub normal rate, on a quarter of a milligram per day of cortisone

Kendall What was their weight?

Ingle They would average about 50 gm

Kendall If they had been 200 gm in weight that would have been 2 mg of cortisone on a weight basis?

Ingle Yes, but no dose of cortisone will sustain the ability of the older adrenalectomized rat to grow Apparently the stimulus to grow is more intense in the younger rat even when he is adrenally insufficient The immature rat loses little or no weight before death from adrenal insufficiency, whereas sexually mature adrenally insuf ficient rats undergo considerable weight loss Sexually mature adrenalectomized rats maintained on cortisone will grow when given pituitary growth hormone

Selye Can you speak of an actual sustaining of growth there because even without any cortisone an adrenalectomized rat, if you put it on salt, will grow some?

Ingle That is true

Selye Have these animals received salt?

Ingle No

Pincus In regard to the effect on the white blood cells in the rat, it would be mostly on the lymphocytes, since the rat has so high a lymphocyte count I would like to recall some experiments that Dr Hechter* published on the effect of adrenal extract on the isolated spleen He found that the isolated spleen would dis charge lymphocytes when perfused with adrenal extract It may be that with the diene you are dealing with the balance between the spleen induced lymphocytotic and a direct lymphopenic effect That there is a balance always in the blood we know very well from a number of experiments It may be that your diene tends to push the balance in one direction and E in another I think a study of the effects of diene on the spleen might be very interesting

Long I would like to point out the importance of the spleen with regard to some studies we have been making on the lymphocytes and eosinophiles I will call attention to the monograph by Hert linger on the adrenal, and on the various types of white cells in

* See reference 5 page 64

the blood Even with injected saline or a wide variety of agents, one of the things which happens is that the eosinophiles rise very rapidly, within 10 or 15 minutes If you remove the spleen the effect is absent So you do have the squeezing effect squeezing the cells out of the spleen

Kendall With the saline?

Long Even with the saline

Thorn Picking up the rats will do it You don t have to give saline

May I go back to one other point? I think this is quite important to establish In the adrenalectomized animal that we used in the past for assay of adrenal extracts we were very careful to limit the salt We know that you can get very good growth curves with salt alone In testing out the efficiency of salt retaining hormone it was necessary to use a diet that had either standard or preferably a low salt intake What I wonder now is if the diet we are using in these animal assays is of such low salt content that the animals would not have the normal growth curve anyway or is there enough salt in the diet to begin with to insure a normal growth curve? Because if we don t have enough salt in the diet to establish a normal curve of growth in a normal animal you could not expect E to give a normal growth in an adrenalectomized animal on the same salt intake It seems to me very important to establish whether E causes enough salt retention to cause a normal growth rate with a normal amount of salt in the diet

Ralli You speak so much about salt Dr Kendall that I was wondering what your standard diet was since there are so many other factors influencing growth I don t want to take away from what I am going to say later but I should think we might discuss the response of animals to various growth promoting factors whether they are normal or adrenalectomized Did you use a synthetic diet?

Kendall A synthetic diet and the salt was adequate for growth

Thorn The salt content was adequate?

Kendall Yes The amounts we used are obviously large They are the rats that received cortisone 2 mg a day Their fur was ruffled they didn t appear normal the loss of weight was not very great but they were poisoned

White I wonder if in the back of everyone s mind isn't the question whether Dr Kendall is too discouraged about the diene to try it clinically

Kendall Just as soon as I get home that is going to be done

White I think that the effects of the described diene on carbohydrate storage and on life maintenance are certainly encouraging

We perhaps know too little about what may influence the blood cell picture, so that the failure of the diene to depress leucocytes would not to my mind eliminate therapeutic possibilities of this compound

Long I wonder perhaps if the conference would not mind shifting the ground a little There is always one problem that arises in a discussion of this kind and that is the whole relationship of the amorphous fraction of cortical extracts or desoxycorticosterone to this problem I, of course and many others are extremely interested in the whole nature of this desoxycorticosterone effect I know Dr Kendall has worked on it and so have the chemists at the Upjohn Company

We have in desoxycorticosterone a substance that is certainly very active in maintaining life, in many ways it appears to act in an opposite manner to the C 11 compounds It might be worthwhile spending a little time on a review of the present situation regarding the desoxy compounds and also what is being done or what might be done in elucidation of the nature of the amorphous fraction

Kendall Did you want me to speak?

Long Certainly, Dr Kendall

Kendall I don't want to have anybody record the statement that I thought that cortisone was the hormone of the adrenal gland and the only one That was furtherest from my mind I don t think *that compounds A and B are delivered from the gland At least it* would be wasteful for them to be delivered because their effect is certainly very small Whether E or F is delivered from the gland may depend upon existing conditions Apparently something affects renal function This is in the amorphous fraction but it has not been identified yet

For the present we feel the urgency of the procurement of cortisone is so great that we do not have the time to study this I think it is a very important thing Although with sufficient salt in the food a patient with Addison's disease can get along *well without the* amorphous fraction He does however need cortisone We have a patient who is a high grade Addisonian She has been treated for over a year now with 25 mg of cortisone every other day that is 12 5 mg a day She lives in Wyoming A little while ago she fell off a horse and broke her leg and went through the experience without any trouble I believe she does take a small amount of desoxycorticosterone in addition to the cortisone It is a very small amount

Conn *Who has treated Addison's disease for any* length of time with E alone?

Thorn We have had some experience with the use of cortisone

alone in Addison's disease. I think Dr. Kendall's figure is a good one. We are shooting for around 10 mg. of compound E per day as a minimum daily maintenance dose.

Long: This is Addison's disease?

Thorn: Yes. That amount of cortisone is quite insufficient for anybody going through even a mild infection of one sort or another. We have had several cases where we have had to increase the dose appreciably during such a period. It may interest you to know that on 10 mg. a day for roughly 45 days the electroencephalogram has not been brought back to normal although the patient feels perfectly well. Perhaps it will take a longer time at that dose level. One hundred milligrams a day for even five or ten days has made a much greater change in the electroencephalogram. We are using the electroencephalogram as one of the most sensitive indicators of adequate physiological response. I would judge, as you have, that 25 mg. every other day would be a very satisfactory dose for most patients with Addison's disease, but we find that most of them will need in addition either desoxycorticosterone in small doses or a high salt intake, since cortisone alone will usually not maintain heart size and blood pressure.

What is the status of the amorphous fraction? I have tried for five years to get my fingers on a little of this. Can't you inject it in animals? Does anyone know what the amorphous fraction does in terms of glycogen in the liver, salt retention, or leukocyte count, all the things that we are interested in?

Long: Dr. Kendall said some years ago that he tested the amorphous fraction for its effect on liver glycogen deposition with negative results. This coincides with our experience with this fraction.

Kendall: Dr. Ingle tested it on the response of muscle.

Thorn: What happens in muscle with the amorphous fraction?

Ingle: It is weakly active. It is more effective than an equal amount of desoxycorticosterone but much less active than cortisone.

Thorn: You feel, Dr. Kendall, that there is primarily desoxy-like action in the amorphous fraction?

Kendall: If you mean by that that the effect is primarily on electrolytes, yes, but it will not in our experience cause retention of salt as does desoxycorticosterone. You can give enormous doses and not raise the level of salt or lower the potassium.

Thorn: In comparison to A or B it is much weaker?

Kendall: They won't do it at all. Not in the rat.

Thorn: They will in man. You get very definite salt retention with A.

Fremont-Smith: A and B?

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Kendall Did you want me to speak?

Long Certainly, Dr Kendall

Kendall I don't want to have anybody record the statement that I thought that cortisone was the hormone of the adrenal gland and the only one That was furtherest from my mind I don t think that compounds A and B are delivered from the gland At least it would be wasteful for them to be delivered because their effect is certainly very small Whether E or F is delivered from the gland may depend upon existing conditions Apparently something affects renal function *This is in the amorphous fraction but it has not* been identified yet

For the present we feel the urgency of the procurement of cortisone is so great that we do not have the time to study this I think it is a very important thing Although with sufficient salt in the food a patient with Addison s disease can get along well without the amorphous fraction He does however need cortisone We have a patient who is a *high grade Addisonian She has been treated* for over a year now with 25 mg of cortisone every other day that is 12 5 mg a day She lives in Wyoming A little while ago she fell off a horse and broke her leg and went through the experience without any trouble I believe she does take a small amount of desoxycorticosterone in addition to the cortisone It is a very small amount

Conn Who has treated Addison s disease for any length of time with E alone?

Thorn We have had some experience with the use of cortisone

administration of large amounts of the C 11 steroids? I think the experience you had at the Mayo's indicates if you give very large doses of compound E, that you do get all these effects that have been described both for the desoxy compounds and the C 11 compounds including a rise in serum sodium and carbon dioxide combining power

Kendall I have arrived at the conclusion that the body has very little power to modify the hormones In a condition like chronic ulcerative colitis where there is a mass of inflammatory tissue, I don t know what that would do but it seems with normal tissue the body cannot change the structure of the hormones very much except to metabolize them completely It cannot use say, the triol 17 20 21 It cannot oxidize 20 to a ketone and use it as such At least the physiologic response indicates that it cannot It would seem almost as though the gland alone could carry on the transformations and deliver the product All the body can do is to use it and metabolize it and ask for more

Pincus I think it is certain from some data that we have published (Hechter et al)* that the gland will oxygenate carbon 11

Kendall The gland will but not the other tissue in the body

Pincus We have done some perfusion experiments which are a little unnatural with tissues other than the adrenal According to these experiments your conclusion, at least tentatively is correct for they will either degrade the material to products beyond our present ability to detect or convert to 17 ketosteroid There are two transformations the gland can effect Oxygenation at carbon 11 and to a lesser degree oxygenation at carbon 21 That immediately gives you so many possibilities that I am afraid it gets rather complicated

Kendall That brings up a very interesting compound in which Dr Gallagher is interested that is the 21 methyl You might think that would be active It is cortisone complete except for the 21 hydroxyl group

Pincus If it is your contention that cortisone is the active compound then a very small amount of that should be transformed

Kendall Suppose you give 100, how much would it transform?

Pincus I can talk about beef gland, a very small amount judging by our somewhat artificial perfusion conditions

Kendall A small amount The amount which could get to the gland be transformed and delivered would seem to be very small Clinically I don t think that this will be useful From the theoretical standpoint it is an interesting possibility

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* See reference 7 page 64

Thorn I have only had A B will in the dog

Kendall E will cause edema in the human if insufficient potas sium is given

White May I ask what is the action of the amorphous fraction on electrolyte metabolism?

Kendall It maintains a nice balance The rat will live on a low salt intake, relatively low It seems to be what Hartman calls the life saving hormone

Selye According to Hartman it has no effect on the potassium, unlike desoxycorticosterone

Kendall That is the point It is not desoxycorticosterone physio logically or chemically I am sure that desoxycorticosterone is not in the gland

Pincus What does the diene do to salt?

Kendall We have not tested that

Sayers Will a large dose of the amorphous fraction induce a *hypernatremia*?

Kendall It will not

Sayers Then it is unlike desoxycorticosterone in that regard?

Kendall Yes

Sayers It seems to me that it is closer to cortisone than to desoxy corticosterone Desoxycorticosterone has a single action—sodium retention regardless of the physiologic needs of the organism Cor tisone on the other hand can induce either sodium retention or sodium excretion depending upon the circumstances This may be taken as evidence in support of the notion that desoxycorticosterone is not a natural hormone

Thorn Cortisone is toxic as far as potassium loss is concerned

Sayers That is correct

Loeb On the other hand there are a certain number of people with adrenal cortical tumors Cushing s syndrome who have exactly the same hypernatremia which you encounter when you give desoxy corticosterone Now of course we don t know whether they are elab orating abnormal steroids or not The fact remains that we have all seen that Anderson pointed that out first I think

Long As Dr Loeb spoke I was trying to jot the questions down in regard to the discussion but perhaps they were a little bit too long I would again like to raise the question about the intertrans formation of some of these compounds in the body For example if you give the large doses of desoxycorticosterone that have been used in some experiments, what are the possibilities of intertrans formation of that material say to a certain amount of C 11 steroid? What are the possibilities of obtaining desoxy compounds after the

Thorn The amount that you can give is so small that it is pretty hard to do chemical extraction I suspect with the aqueous type of desoxycorticosterone where you have a short lived action you might give larger quantities and not produce extensive salt retention and make a good metabolic study out of that That is a possibility today which we did not have in the past

Conn Returning to the question of transformations within the body whether they are somatic or adrenal cortical and then to Dr Loeb s remark that it is true that with large doses of cortisone one can produce potassium diuresis a desoxy like effect we have been studying sweat electrolytes for some time and have convinced ourselves that when desoxycorticosterone is given one gets a very sharp reduction in sodium and chloride concentration of the sweat Recently we found that with large doses of cortisone too, one can produce a sharp reduction in sweat sodium and chloride again a desoxy like effect, that this occurs at a time in a normal man when the sodium of the serum rises and potassium of the serum falls Whether this represents a competitive situation between E and desoxycorticosterone or whether it represents a transformation of some E to a desoxy like compound is still open to question

Selye In this connection it is interesting that cortisone will produce hypertension and glomerular changes in the kidney such as were previously produced with desoxycorticosterone as long as the animals are specifically sensitized for the salt metabolism effect That is to say in partially nephrectomized animals obtaining a high sodium diet cortisone acts somewhat like desoxycorticosterone This might obviate some of our difficulties in connection with the interpretation of the pathogenesis of disease of adaptation (whether or not desoxycorticosterone as such is produced in the body) since so much depends upon the conditioning effect of intermediate metabolism Under certain conditions even the so called sugar active' gluco corticoids can produce hypertensive changes although they rarely, if ever produce periarteritis

A particularly remarkable sideline of this is that if you give threshold doses of desoxycorticosterone which are almost inactive and at the same time give corti one then the changes develop so rapidly that you produce a condition of hypertension and nephro sclerosis within 15 days in the sensitized animal In other words in rats sensitized to the mineralo corticoid effect by partial nephrectomy and a high sodium intake the toxic effects of desoxycorticosterone are further aggravated by simultaneous cortisone treatment Incidentally Reichstein s compound S (desoxycorticosterone), which is undoubtedly a normally occurring mineralo corticoid also produces

Ralli Does a pathological state in the body such as disease influence the ability of the adrenal gland to transform such substances? For example, patients with diabetes are not prone to develop rheumatoid arthritis this has been noted by many observers and rheumatic fever is seldom seen in diabetic children I wondered if such observations would indicate that in these diseases the adrenal is hyperfunctioning and whether situations might exist which accelerate or retard the transformation of the steroid hormones?

Long In diabetes don't you have, or suspect that you already have, an absolute or relative hyperactivity of the adrenal cortex?

Pincus We have studied it in alloxan diabetic rats There is very good evidence of increased adrenal secretion in alloxan diabetic rats (Pincus, Scola and Elmadjian)*

Ralli Is there any way of measuring the amounts of the adrenal cortical hormones normally present and which therefore could be considered amounts sufficient to prevent these collagen diseases?

Pincus We studied adrenal cholesterol which is quite low in these animals and attempted to restore it to normal level in the hope of thereby controlling the diabetes

Bloch I would like to come back to the problem of hydroxylation There seems to be some contradiction between Dr Pincus' results with the perfused beef gland and the observations with humans which indicate that compound S or desoxycorticosterone are not converted to 11 hydroxy compounds Perhaps species differences are involved or the administered compound may never reach the site of conversion But evidently enzyme systems are present which can introduce a hydroxyl at C 11 of the ready made steroid

Pincus The perfused gland will convert S to F (Hechter et al)** The species difference is something that is possible but not problematical since for example, E and F have been found in human urine The experiments of Vogt*** are very instructive because she has found very rapid disappearance of parenterally administered corticosteroid It may be there is a competition between soma and adrenal in which the catabolic activity of the soma is overwhelmingly large She found—I don't remember the exact figures—the destruction of something like 99 percent of the administered steroid by the somatic tissue

Bloch Is there any difference in the excretion pattern after the administration of corticosterone and desoxycorticosterone?

Thorn In man?

Bloch Yes

* See reference 14 page 64
** See reference 8 page 64
*** See reference 18 page 64

Sayers You say it is conceivable Has it been studied, *i e*, in relation to liver disease?

Thorn I think those would be the diseases to study rather than the more diffuse diseases

Long As I recall, when we recessed we were discussing the question of the metabolic transformations of the adrenal steroids Does anyone want to continue on this point?

Thorn May I ask this question Is it conceivable whereas compound E can be degraded to desoxycorticosterone, that in general, desoxycorticosterone might be synthesized to compound E? I mean from the chemist s viewpoint, what are the probabilities?

Kendall I think they are very small

Pincus I would second that We tried to get it done by isolated tissues with no indications of success

Long I take it then as far as the gland is concerned it can convert the 11 desoxy compounds into 11 oxygenated compounds but the reverse reaction has not so far been shown?

Kendall From all our evidence it is the 11 hydroxylation that the glands perform Dr Mason, after extracting hundreds of liters of urine of the patients that received compound E, was able to isolate a few milligrams of compound E but found also that it contained some compound F It is very easy to test for compound F because of the green fluorescence with sulphuric acid A small amount will show up From hundreds of liters of urine a very few milligrams of compound E were separated

Long Dr Kendall in these cases where the individuals have been given ACTH to stimulate the natural secretion of the gland may I ask whether under those conditions Dr Mason has isolated mostly compound F from the urine?

Kendall Yes

Long Does he find any compounds or substances or group of substances with the activity of the amorphous fraction after treatment with ACTH?

Kendall Physiological tests have not been made

Long Do you find the desoxy type of activity in the fractions he isolates?

Kendall No physiologic tests were made

Pincus We have extracted normal human urines and assayed for electrolyte active corticoid but have not examined pathological urines (Marcus, Romanoff and Pincus)*

See reference 10 page 64

periarteritis nodosa, nephrosclerosis and hypertension in sodium sensitized rats

White I have one question which relates to the problem of what tissues do with the steroids Perhaps more appropriately it belongs with Dr Loeb's discussion I was wondering what the urinary excretion of the corticoids in the wide variety of clinical circumstances that have been treated with cortisone tell us with respect to what those tissues in those diseases can do with cortisone Is my query clear?

Long Yes You want to know if in the individual rheumatoid arthritic the excretion of the cortical compounds is any different from normal

Thorn I was interested in Dr Kendall s remarks about the large doses of compound E given to children without toxic effect I would assume when one gave cortisone to a child particularly with disease that it would be a debatable question as to how active that adrenal might be and that actually the amount of cortisone required to suppress that adrenal might be just as great as it would be in the adult and that the dose of cortisone would not become toxic until you had topped the adrenal atrophy It would surprise me if a child did not require almost as much as an adult, if not more, to cause complete atrophy The initial level of the adrenal steroid varies widely in the disease states which we have studied Therefore before giving compound F one should know the initial level We don't have enough data but our figures would support Dr Kendall exactly and that is, that 100 mg of E gives you about 2 to 3 mg of 17 ketosteroid increase and with the doses as large as we have used one does not approach the 17 keto secretion or the 11 oxy secretion produced by ACTH

White Do we know what proportion of administered cortisone having an established base line, comes out as the so called corticoids in these various conditions?

Kendall That is followed very closely

White What would you say is the capacity of diseased tissue versus normal tissue to transform administered cortisone into corticoid metabolites?

Kendall In all cases at least 96 percent never appears in the urine

White Under any circumstances?

Thorn Less than 10 percent If you happen to get a rheumatoid with amyloid disease of the liver conceivably the whole thing might be changed

possibility of elaboration of small but very effective amounts of this type of substance

Long On the other hand, 50 mg of the C 11 compound with only a fiftieth of the salt retaining activity would make the problem more complicated, would it not?

Loeb Yes

Long You have no desoxycorticosterone in 50 mg of cortisone but still obtain a salt retaining effect

Pincus I think the answer might very well come from ACTH administration experiments If ACTH causes a really large increase in the urine then one can partition the steroids adequately

Long Dr Kendall says Dr Mason does not find any appreciable amounts

Pincus Has he tried to find activity? He just tried to isolate products

Kendall Yes he did try He tried to separate crystalline material that he could identify

Pincus The amounts present must be extremely small because in using a new color reaction rather specific for 11 desoxycorticosterone (Pincus and Romanoff)* indications of only microgram amounts per day have been found I think Dr Loeb is placed in a good position for recognition unless after the administration of ACTH perhaps to certain particular types of cases we obtain better indications

Thorn It seems to me that it would be interesting to return to Dr Loeb's earlier remark In the Cushing's patient we have the spectrum of normal sodium to high sodium with edema which of course could easily be due to an abnormal compound We have something there which none of us has seen with compound E, namely, an elevation of the sodium level above normal

Long Didn t Dr Sprague say that when he gave large doses of E of the order of 200 mg a day he observed sodium retention and an increase in CO combining power of the blood and presumably water retention?

Kendall A drop in chlorides

Thorn That may be the distinguishing feature Compound E in excess may drop the chlorides I have never seen low chloride with desoxycorticosterone

Selye Marked hypochloremic alkalosis is regularly obtained also if you give high doses of desoxycorticosterone to rats In fact this is the most important change in sodium chloride metabolism

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See reference 15 page 64

Long Have you tested this for any salt retaining effect?

Pincus What we do is use essentially the test that Dorfman et al * described using radiosodium We used the flame photometer for Na and K measurement The amount of salt retaining materials in human urine is extremely small We can find an equivalent of somewhere between five and fifteen micrograms of desoxycorticosterone per day, and that is doing pretty well

Sayers Dr Pincus, aren't you going to get into some difficulties if you attempt to say something about the chemical structure of these compounds on the basis of sodium retention?

Thorn I would also like to raise that question I don't know of a biological assay set up today that is worth anything in terms of specificity of desoxycorticosterone It is now evident that salt retention is shared by most active adrenal steroids I don t think we can come at it from the salt or water retaining assay

Pincus We have run a number of pure steroids and adrenal extract in this test By far the most active compound in salt retention is 11 desoxycorticosterone

Sayers Dr Pincus would you not have to carry out some chemical fractionation of your urinary extract?

Pincus I ought to give you some more details because this is not yet published What we did was take the urinary corticoid and do the Kendall Reichstein partition between benzene and water of the neutral lipids We found as you and Dr Thorn would probably expect that salt retaining activity was present in both the benzene and aqueous fractions but that the amounts present in each were not particularly different They are about the same in each fraction You would therefore conclude provided this partition is effective and where we are dealing with microgram amounts that is always a problem, that there were both desoxy like and more soluble types of steroid present

Loeb I still would like to think there was something like desoxy in the adrenal cortex After all one can get fairly significant salt and water effects in the Addisonian patient frequently with one milligram of desoxy a day Thus one is dealing with an activity as far as salt and water are concerned of quite a different order of magnitude than that we see with the 11 oxy compounds If one only has, say, the equivalent of one milligram of desoxy like material elaborated a day, the chances of discovering or finding any of it or its degradation products I think would be almost hopeless and I would wonder if the fact that you did not find it would in any way exclude the

* See reference 1 page 64

Fremont Smith May I make a suggestion? I wonder whether the kinds of statements that have come out of this meeting could not be tabulated We have reached a point where it would be extremely useful if it has not been done already, to prepare a table or series of tables which would deal with the compound the dosage the species and the effect whether the animal was normal, or adrenalectomized and insert specific disease syndromes where we know them Such tables would, I think show certain gaps right away They might, when we put them together, show certain correlations which had been overlooked

Ralli I think it is an excellent idea Such a table should also include the species and number of animals used We accept at times as facts data derived from the study of a small series of animals which later observations prove to be incorrect

Fremont Smith Could we not have footnotes which would both give the number of animals and any other pertinent data that could be squeezed in and the reference? Then this would have to be something that would be cumulatively changed and very rapidly as time goes on If this idea is any good the tables could be brought up to date every year

Ralli What you would have soon would be a map that would cover the wall

Fremont Smith I am visualizing a series of nomograms which would really build these things together Perhaps that is a dream Doesn t the very complexity of the problem demand that we get down in tabular form what we now know?

Thorn I think there is very good agreement I suppose it would be an interesting thing to have an available summary

Ralli May I ask one question? Going back to Dr Pitts who is unduly modest—and incidentally I notice that all the kidney physiologists back water when they are questioned—in the injection of hormones that have marked effects on sodium and chloride metabolism is there any effect on the pituitary with a consequent effect on tubular reabsorption of water or salts? Would this be a direct effect on the kidney or an indirect effect due to stimulation of the posterior pituitary hormone? Does not the amount of salt in the animal influence the manner in which the kidney handles the salt excretion?

Pitts The fact which has completely baffled me is that the renal tubules of the adrenalectomized dog when presented with an ordinary load of salt and water absorb and conserve it less completely than do those of the normal and yet load that animal with salt as Dr Thorn pointed out and the tubules overabsorb both salt and

Pincus In the adrenalectomized rat?

Selye Yes

Sayers You get hypochloremic alkalosis?

Selye Yes

Sayers May I interject a point here in regard to dosage? The problem of dosage has not been sufficiently explored particularly with regard to cortisone It seems to me what is needed here is a rather extensive study of electrolyte balance in animals and man with varying doses of cortisone in order to answer some of the questions that have been posed

Another point which comes up is the question of the actions of these hormones in a situation in which they are actually inducing hypercorticism in contrast to situations in which a state of eucorticism exists May we expect to get electrolyte responses in states of hypercorticism which differ from those obtained when the tissues are exposed to quantities of cortical steroids just sufficient to meet physiological needs?

Is there a difference in electrolyte response between maintenance doses of compound E and doses of compound E which will induce hypercorticism?

Thorn What was the last part of that question?

Sayers Does anyone know whether doses of cortisone which *induce hypercorticism —such as doses now being used in rheumatoid* arthritis—induce continuous retention of sodium?

Thorn You get a reversible effect I take it you all observed that in a week or two the salt retention shifts to a salt loss

Sayers You get escape We get the same thing with ACTH Initially we get sodium retention then sodium loss

Thorn We know that 100 mg of compound F daily may eventually produce Cushing s syndrome You could help yourself in the desoxycorticosterone problem by dropping down under 100 mg of compound E in Addison s disease and see what ultimately happens to salt retention In other words if we could carry an Addisonian for an indefinite period on 50 or 60 mg to see what would happen to salt retention that might lead us somewhere

Long We have had a lot of discussion on the salt and water effect of adrenal steroids This involves the renal tubular mechanism We have Dr Pitts with us and we might ask him to comment All seem to agree that one of the effects of these adrenal steroids is to affect the renal tubular mechanism Have you anything to bring to us on that point?

Pitts Nothing at all except I think further mystification I can contribute nothing but confusion to the problem at the moment

reabsorption is not apparent until perhaps a half or three quarters of an hour As a consequence if the potassium output is markedly increased in the first 15 minutes, the excretion of chloride will be elevated in proportion, even though the excretion of sodium is unchanged So just from the time relationships we would assume that the absorption of potassium is independent of the absorption of sodium, and that the effects of the hormones on the excretion of these two ions are manifestations of two independent renal actions

Loeb Is that on a milli equivalent basis?

Pitts Yes Just to give you an example these animals might be excreting 100 micro equivalent of sodium per minute perhaps only 10 or 15 micro equivalents of potassium Give them desoxycorti costerone intravenously and you might boost the potassium up to 100 micro equivalents per minute within the first 15 minutes without affecting the excretion of sodium at all The excretion of chloride would increase in proportion to the increase in excretion of potassium

Long Is it feasible to study these effects in isolated kidneys where the organs would not be influenced by what is taking place in the other body fluids?

Pitts I don t know We have never done it As far as I know, no one has I don t know how long you can preserve a pump lung kidney preparation in a very reasonable state Of course Winton and others have studied such preparations with respect to water and chloride excretion but the question of reabsorption has never been considered adequately Shannon maintains that the tubules lose their impermeability to creatinine in such a preparation Whether one can measure glomerular filtration rate as a preliminary to meas uring tubular reabsorption is questionable However it might be worth trying

Ralli Does the plasma flow change at all in these experiments?

Pitts Yes but not very significantly

Selye What about the diuretic effect of desoxycorticosterone which is always very pronounced if you give large enough doses? This effect is increased by hypophysectomy We have removed the whole pituitary to establish this and cannot say whether the antidi uretic action of the pituitary is an anterior or posterior lobe effect We ought to find out whether the compensatory secretion by the posterior lobe could normally inhibit the action of desoxycortico sterone and similar compounds

White That point is well taken in view of the work of Goodman and Gilman in New Haven some years ago demonstrating a response of antidiuretic hormone secretion to sodium chloride and water content of the animal either as produced by the reduction of salt

water The capacity to reject is no less affected than is the capacity to conserve One can correct fairly rapidly the incapacity of the tubules to salvage salt and water at normal loads by giving desoxycortico sterone or adrenal extract One cannot so readily correct the incapacity to eliminate salt and water, if indeed one can affect it at all Why should a renal tubule presented with a normal or low load be unable to reabsorb it as completely as does the normal, and when presented with a large load overabsorb it and be unable to eliminate it as rapidly as does the normal?

Long Is there a 'T' maximum for sodium?

Pitts As far as I know there is no 'T' maximum for sodium

Thorn Elimination is decreased I don t know where the point is

Pitts Our experiments were rather simply conceived but did not provide a simpler answer by any manner of means If you infuse a normal dog with hypertonic saline, say 3 5 percent sodium chloride, the percent of the filtered sodium reabsorbed by the renal tubules may gradually drop to 75 or 80 Under normal conditions, that is prior to salt infusion some 99 percent is reabsorbed That same animal after adrenalectomy, infused with hypertonic saline will reabsorb 90 to 95 percent of the filtered sodium We have never gotten them below 90 percent In other words the normal animal can stop reabsorbing sodium, if you want to look at it that way, with a great deal more facility than can the adrenalectomized

Thorn If you are dealing with the untreated, adrenalectomized animal, the amount of intracellular fluid available is large compared with the extracellular fluid What this does in terms of affecting the salt load is not known

Pitts Our animals were maintained prior to the experiment with 0 6 percent sodium chloride ad lib They were healthy and in good shape

Thorn But their water content is higher?

Pitts That would be higher

Long What is the relationship of that and the failure to clear potassium? It seems to be an equally striking effect in the adrenalectomized animal

Pitts You mean the overabsorption of sodium and the failure to clear potassium?

Long Yes

Pitts Very recently we said we thought they were independent largely because of differing time relationships If you give an intravenous dose of whole adrenal cortical extract or give desoxy corticosterone in sesame oil emulsified in water, you observe increased potassium excretion the first 15 minutes Increased sodium

venously into the dog The different routes of injection may have had some influence on the results

Loeb It is active in the hypophysectomized animal

Ralli In the hypophysectomized dog?

Loeb In diabetes insipidus

Ralli The material which we eluted on a permutit column from a urine concentrate was not antidiuretic either in the hypophysectomized dog or in normal dogs under a water load In each instance the injections were given intravenously and we are now testing the extracts in the dog intraperitoneally as well as intravenously It was Dr Heller who drew our attention to the fact that the method of administration might account for the differences between the rat and the dog

Thorn If you go back to the Addisonian patient there is a nice correlation with the Gaunt test whatever it means Given a water load, *the Addisonian is not unable to excrete water it is just a* delayed diuresis In other words your test is based on a time interval which a load of water requires for excretion after a standard administration If you follow that patient into the afternoon when he has his delayed diuresis the antidiuretic substance drops in the serum Such experiments were done by Dr Alexander Slessor in my laboratory on desoxy maintained Addisonians They were given a small dose of 2 mg per day They did show an increased antidiuretic assay in the morning As the day passes on when the urine begins to flow, the antidiuretic substance in the urine and blood begins to fall at the time they have their diuresis

Ralli We have had the opportunity of studying several patients with chromophobic tumors of the pituitary Two have had adrenal cortical insufficiency and one patient also had diabetes insipidus The latter resulted in quite a complicated situation This patient developed diabetes insipidus first and then went on to develop adrenal cortical insufficiency which required treatment with DOCA and adrenal cortical extract The administration of DOCA and salt aggravated the diabetes insipidus We have assayed his urine for its antidiuretic effect after taking pitressin under a variety of conditions When he took the pitressin at midnight and did not void until 8 A M the urine being held in the bladder for this period of time we got no antidiuretic effect on rats or dogs When we injected pitressin and collected the urine 2 hours after the injection the urine was antidiuretic in both test animals and in the dogs it was given intravenously This suggested to us that the antidiuretic substance in urine may be a large molecule which undergoes some changes probably splitting off some fraction and when this happens

and water intake or excessive sodium chloride intake

Thorn We can get some information on that It is known that the patient with Addison s disease has both a high blood level of antidiuretic substance and a high urinary level It seems possible that the Kepler Power water test in such patients reflects this excessive antidiuretic principle If you give desoxycorticosterone to the patient you get no change in antidiuretic level in the urine This is using the technique of Gaunt If, on the other hand, you give compound E, you immediately restore to normal the antidiuretic levels in urine The problem is whether this is suppression of the posterior pituitary or inactivation of the posterior pituitary hormone at the peripheral level

We took one patient with diabetes insipidus and kept that patient on a standard amount of pitressin which did reduce his spontaneous diuresis from about 12 liters to 4 liters Then giving 100 milligrams of compound E per day we did not produce a diuresis Insofar as that single experiment is worth anything, it would appear that corti sone did not affect the peripheral activity of posterior pituitary hormone This leaves as modes of action either suppression of the hypothalamus or the posterior pituitary by cortisone

Pitts I think I am quoting Gaunt correctly in his recent experi mental work on adrenalectomized rats Heretofore he has never been able to restore water excretion to normal with desoxycortico sterone However in recent experiments he has but the doses are of the order of 10 mg Under such conditions he does not find any drop in the serum antidiuretic hormone level at the time that that animal is capable of giving a normal response to a water load, which would indicate that DOCA has a diuretic effect *per se* independ ent of any other action it may have

Loeb In our own observations if you give DOCA with a comple ment of salt of say, 0 9 percent in the drinking water you have this increased turnover of water If, on the other hand the animal is given the same dose of DOCA on the salt poor regimen there is no increase in other words the increase is dependent on the sodium intake

Pitts I should state that in the dog we have never seen any indi cation of a diuretic action of DOCA such as that described by Gaunt in the rat

Ralli Apparently the dog and rat react somewhat differently In some of the work that we have been doing with the antidiuretic substance in patients with liver disease we found that the extract from the urine of these patients produced antidiuresis when injected intra peritoneally into the rat it was not effective when injected intra

adrenalectomized animal led to coma and disappearance of cortical potentials We explained these results on the basis of a difference in the rate of the fall of the blood sugar in the two groups since the blood sugar curve fell more rapidly in the adrenalectomized than in the adrenodemedullated group However I wonder now whether this is a good explanation Perhaps the result is caused by changes in the electrolyte content of the brain McQuarrie and collaborators showed some time ago that the injection of desoxycorticosterone lowered the potassium concentration of the brain The question I wanted to ask is whether there is any evidence that there is a change in the electrolyte content of the brain in the adrenalectomized animals also what is known about the effect of the various adrenocortical hormones or steroids which were discussed here today on the electrolyte content of the brain

Loeb Dr Long some people in our place some years ago did tisue analyses on most of the tissues of normal in contrast to desoxy treated dogs and when it came to the brain, Dr Gellhorn they got into such a mess with all the lipid material that they did not know what their analyses meant With all the lipid material the difficulties of analysis were pretty great We have no definitive answer as to changes in intracellular potassium in the brain at all by virtue of the technical difficulties we encountered

Sayers Drs Woodbury and Davenport have conducted experiments in order to make a correlation between changes in tissue and extracellular electrolytes as they relate to electro shock convulsive threshold of the central nervous system (Woodbury D M and Davenport V D Brain and plasma cations and experimental seizures in normal and desoxycorticosterone treated rats *Am J Physiol* 157 *234* (*1949*)) They have come to the conclusion that the brain is rigidly fixed in its electrolyte concentration and that DCA does not change brain potassium or sodium at a time when marked changes occur in plasma sodium and potassium This is not in agreement with the work of McQuarrie and coworkers (Ziegler M R Anderson J A and McQuarrie I Effects of desoxycorticosterone acetate on water and electrolyte content of brain and other tissues *Proc Soc Exper Biol & Med* 56 *242* (*1944*))

Pincus Normal or adrenalectomized?

Sayers Both normal and adrenalectomized animals

Thorn I think you could show some change in threshold in your adrenalectomized animal with an intravenous injection of compound E or F before there was any chance to have appreciable electrolyte loss in the urine or a change in overall distribution within tissues

The interesting thing with compound E is that it is not particularly

the substance may not be active in the dog It seems reasonable to infer that the antidiuretic principle as secreted by the gland is changed in the body and is excreted in a somewhat different form in the urine This form may certainly be different than commercial pitressin which is extracted from the whole gland

Long As far as I know these effects of desoxycorticosterone occur in hypophysectomized animals

Thorn In Gaunt's?

Long Yes Although this may be a complication, perhaps it explains all the effects of the desoxycorticosterone

Thorn In Dr Ralli's patient salt alone might be expected to activate the diabetes, so one would be up against the question as to whether the salt did it or the desoxycorticosterone

Ralli In this instance we did not give salt alone for a while Then we reduced the salt and gave him the desoxycorticosterone

Thorn Both made him worse?

Ralli Yes

Ingle This will show my complete ignorance of the fundamentals of renal function In excreting the high sodium load does the kidney do work in getting rid of sodium?

Pitts The osmotic work that is done by the normal kidney in eliminating a large load of sodium becomes negligible If you want to calculate osmotic work according to the equations of Rappaport or Newburgh it does not take a great deal of osmotic work on either sodium or chloride to eliminate a large load That is simply because the animal puts out urine which is practically isotonic with plasma On the other hand, the adrenalectomized animal, given a comparable sodium load puts out less sodium and chloride but puts it out in a much more concentrated solution hypertonic to the plasma and actually does more osmotic work in eliminating that given load than does the normal animal

Gellhorn I wonder Mr Chairman if we have not come sufficiently close to the brain since the pituitary mechanisms have been mentioned to raise a neurophysiological question to which some of you may have an answer Several years ago we studied the effect of insulin on blood sugar EEG and general behavior in adrenalectomized and adrenodemedullated rats (Arnett V, Kessler, M and Gellhorn E The role of the adrenal cortex in preventing convulsions *Am J Physiol* 137 *653* (*1942*)) It was found that convulsions occurred at a much higher blood sugar level in the adrenalectomized animals than was seen in the adrenodemedullated animals In addition it was found that adrenodemedullated rats frequently showed no changes in the EEG at a blood sugar level which in the

other electrolytes except chlorides and found these unchanged in desoxycorticosterone treated animals as long as there was no brain edema, but if you treat them long enough you invariably get brain edema at least in the rat I think this is a precursor of periarteritis nodosa of the brain and of course with local vessel lesions, the chloride content of the brain increases very much This is probably only due to the fact that there is more extracellular fluid (edema) and that is naturally rich in chloride We became interested in this in connection with the anesthetic effect of various hormones and the effect on the nervous system in general While a large series of steroids (including progesterone and even including some hormonally inactive compounds like pregnanedione) caused first a state of excitement then a state of depression and then anesthesia, only desoxycorticosterone changes the brain electrolytes Even this compound acted only when obvious brain edema had ensued

Long We have wandered a little far away from the comments of this morning We still have before us this question of the remarkable differences between DOCA and the C 11 steroids There are certain similarities perhaps but by and large they still seem to have very different effects

Thorn May I ask a question at this point of Dr Kendall? What is the pituitary inhibiting activity of compound S and do we have anything in the previous biology of S to make us think that it might be anything like compound E in terms of inhibiting effect on the pituitary? Everybody went to S with a great deal of hope On what was the hope based?

Kendall The formula

Thorn I take that for granted but I wonder has anybody shown for instance that S is any better than desoxycorticosterone as a pituitary inhibitor?

Kendall In suppressing the pituitary?

Thorn Yes It would be a nice screening test for E like compounds Who has tested it?

Sayers I would like to have some of it to try because undoubtedly it is more water soluble than DOCA

Kendall Not much a little

Bloch Looking at the structure of desoxycorticosterone and the 11 oxy compounds the introduction of the hydroxyl groups should change the solubility quite markedly and it also provides the possibility for conjugation I want to ask whether there is any evidence that any of the hydroxy groups in the cortical steroids give rise to conjugated forms, such as glucuronides

Kendall Not to my knowledge but there is an interesting point

protective against insulin although the threshold for hypoglycemic symptoms is definitely elevated Those figures are not statistically significant but certainly if you follow them from patient to patient you find a restoration of the threshold for symptoms of hypoglycemia At the end of a 24 hour fast the individual with compound E may not have his blood sugar level elevated but will nonetheless fail to show hypoglycemic manifestations at a blood sugar level identical with the one previously accompanied by marked symptomatology Of course, I think our early experiments along the same lines with adrenalectomized dogs at Hopkins did show that the adrenal extract would raise the hypoglycemic threshold in the adrenalectomized dog

We had a patient with diabetes and Addison s disease who got hypoglycemic manifestations when the blood sugar dropped from *400 to 300* *We can drop from 400 to 300* with *E and not get the* hypoglycemic manifestations even though the rate of fall is about the same I don t think it is the rate of fall in the adrenalectomized animal alone It is obviously another factor related to improved carbohydrate utilization with cortisone

Gellhorn I know that Engel (Engel R Thesis University of Minnesota (*1949*)) in a recent Master s thesis from McQuarrie's department did not confirm McQuarrie s earlier studies (Ziegler, M Anderson J A and McQuarrie, I Effects of desoxycorticosterone acetate on water and electrolyte content of brain and other tissue *Proc Soc Exper Biol & Med* 56 *212*, (*1944*)) according to which injection of DOCA lowered the K content of the brain I agree that in general the brain is chemically extraordinarily stabilized but still there may be minor changes which due to technical difficulties have not been found yet

Conn *With regard to the electrolyte changes didn t you* Dr Thorn some time ago get the same kind of a change with desoxy corticosterone in Addison s disease that is a tendency not to have hypoglycemic manifestations at the same blood sugar level in a patient getting desoxycorticosterone?

Thorn I don t think so As a matter of fact in overall carbohydrate tolerance we always felt that the desoxycorticosterone enhanced the hypoglycemic effect in terms of threshold What we did show in 1939 was that the absorption of glucose from the gastrointestinal tract was improved under desoxycorticosterone which we took to be an over all effect on electrolytes and better hydration

Selye I wonder whether all these contradictory findings concerning brain electrolytes are not explicable by the development of brain edema in certain cases and not in others? We have not done any

other electrolytes except chlorides and found these unchanged in desoxycorticosterone treated animals as long as there was no brain edema, but if you treat them long enough you invariably get brain edema at least in the rat I think this is a precursor of periarteritis nodosa of the brain and of course with local vessel lesions the chloride content of the brain increases very much This is probably only due to the fact that there is more extracellular fluid (edema) and that is naturally rich in chloride We became interested in this in connection with the anesthetic effect of various hormones and the effect on the nervous system in general While a large series of steroids (including progesterone and even including some hormonally inactive compounds like pregnanedione) caused first a state of excitement then a state of depression and then anesthesia, only desoxycorticosterone changes the brain electrolytes Even this compound acted only when obvious brain edema had ensued

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that the hydroxy compounds are less soluble than the keto compound in every instance that I know That is very true of those with the C 3 hydroxyl All the compounds with the C 3 hydroxyl and no double bond come out first from the water solution The compounds with an unsaturated ketone are appreciably more soluble in water Also compound A without the OH at C 17 is more soluble than compound E with it there, so that the generality of the increase in solubility of the hydroxyl groups does not follow in these compounds

Thorn But F is much more soluble than E

Kendall F is more difficult to extract from water with chloroform yes

Bloch Many steroids are transported in tissue fluids as conjugates, such as fatty acid esters or glucuronides Is it possible that the amorphorus fraction represents hormones in conjugated form?

White May I ask in that connection is the evidence at the present time that the adrenal cortical steroids like certain of the estrogens, are transported in conjugation with blood proteins? Is there any evidence at the present time on this point in relation to the adrenal cortical steroids?

Kendall I don t know of any

Long I don t think Dr Vogt ever tried that She gave the whole plasma She never tried the extracts after precipitation of proteins

Pincus There is some evidence from urinary extraction that there must be some conjugation because if you decrease the pH at which the corticoids are extracted from urine you can get an apparent increase in extractable corticosteroid There is definitely much less extractable at pH 4 than at pH 1 (Heard et al)* Also Dr Venning** has reported that maintaining urine at an acid pH over a period of several days will result in further release of hormonally active corticosteroids So I don t think there is much question that corticosteroid conjugates are formed in the body We have recently (Pincus and Romanoff)*** reinvestigated the urinary corticosteroids and find all the published excretion figures including our own are off by a factor of about three If you use the proper hydrolytic conditions you get not one to three milligrams a day but more likely five to nine a day So I don't think there is much doubt about the conjugation in the urine As to what happen in the blood I don t know of any really informative experiments

Kendall You refer to urine after ACTH or corticosterone?

Pincus Just normal human urine Also in an experiment done

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* See reference 4 page 64

** See reference 17 page 64

*** See reference 15 page 64

recently with glucuronidase, in other words, using enzymatic hydrolysis, Engel* found an increased yield over that obtained by ordinary methods of extraction Since not only reducing and formal dehydogenic lipid but also biological activity increase after acid or enzymatic hydrolysis the suggestion is unavoidable that the 21 hydroxy group is conjugated

Bloch Is there any indication as to the excretion of cortical products by the fecal route? The normal route of excretion for the end products of cholesterol and bile acid metabolism are the feces and the normal concentration of cholesterol in urine is only about 1 mg per liter

Kendall Nothing

Long As far as I know there have been no such experiments with the cortical hormones

White This may explain the 90 percent so called disappearance of administered cortisone

Ingle Hasn't the Jefferson group done a little along this line Dr Pincus?

Pincus What little information we do have relates to 45 pregnenolone administered both orally and parenterally to rabbits (Pearlman and Pincus)** The feces yielded no identifiable metabolite nor even pregnenolone itself The urine yielded a little pregnanediol

Bloch I think the unsaponifiable fractions from the tissues and feces have never been thoroughly studied except the adrenal and perhaps testes but I believe if one made a comparably exhaustive study of steroids from the liver one might find a whole number of compounds one never suspected For instance the unsaponifiable fraction of liver contains about 70 percent cholesterol a little bit of aliphatic alcohols and some hydrocarbons but the major part of this remaining non cholesterol material is unidentified and it really has not had enough attraction because the liver is not an endocrine organ

Long Dr White will remember we had a Dr Horwitt who studied the kidney steroids This work was not published but the general conclusion reached was very much in line with what you say Dr Bloch that there is a large group of compounds in the unsaponifiable fraction that might be well worth investigating At that time in view of the close relationship of the kidney tubules and the adrenal cortex we were interested to see if there was any adrenal cortical activity in such kidney extracts We worked up about a thousand pounds of kidney but the material was never properly characterized

* See reference 2 page 64
** See reference 12 page 64

except to say there were present a large number of steroid compounds

Pincus I think Dr Pearlman's* published paper on the bile might be mentioned He definitely found non saponifiable compounds, not identified particularly as adrenocortical derivatives, but he did report to the International Biochemical Congress (1949) in England the isolation of what is probably Δ9, 11 progesterone That is the nearest thing that we have gotten to a sort of adrenal corticoid substance He has, however reported very briefly that there are a number of alcoholic steroids in bile, probably having a number of hydroxy functions which he has been unable to characterize I agree with you I think that the bile and the liver both should be explored for the neutral steroids Except for Dr Pearlman s work I know of only one other very brief publication that was from England I have forgotten the author s name for a moment His exploration as a matter of fact was rather small but even there he obtained one new compound, a Δ16 androstenol

Long I have one practical question about which I should like to ask Dr Kendall for his advice and guidance I think it would be of benefit to the whole group Time and again this morning we have discussed the difficulties that are inherent in the solubility of these compounds, in making these various biological tests of their activity I think perhaps some time you could use your good influence with the Merck Company to produce the hemi succinate of compound E which is a highly soluble substance Certainly anyone trying *in vitro* work is up against the problem of the insolubility of these substances

Two or three years ago Dr Sarrat made some of the hemi succinate A and we found on the glycogen deposition tests that the effects were certainly greater than those produced by the material dissolved in oil Not only was there a much greater degree of activity but also there was uniformity of response from animal to animal and anyone who has used the glycogen deposition test knows that you get a high degree of variability even with active compounds when they are given in oil

Kendall What was the dose?

Long They were given in the neighborhood of 2 mg per 100 gm

Kendall I would like to reply to your question Dr Long I think that point is very important I regard it as perhaps the most important reason why we were able to show the effect of compound E on arthritis a result that we might well have missed When it came to preparing this material I started out very much of a freshman and thought I could write to various companies and get information I tried to and could not get any help whatsoever so then I started in An Erlenmeyer flask was indented lengthwise and was

* See reference 13 page 64

then rotated with 0 9 percent salt cortisone acetate and balls of various composition First with glass beads they did not work at all Then we used steel ball bearings which worked fine, except that the steel went into solution and produced a chocolate precipitate We tried nickel plated steel balls stainless steel balls nickel balls silver nickel alloy balls of several different sizes Then we heard about the hardest compound known tungsten carbide So I ordered some tungsten carbide balls Those ball bearings cost several dollars each, but just before they came we had another experience We ground cholesterol in the flask and contrary to the other compounds we found that it floated The specific gravity of cholesterol is greater than water so it should have gone to the bottom Then it became evident that a film of gas on the particles was very important Some Tween 80 was added and the finely ground cholesterol dropped to the bottom That gave a clue as to what might be the way to do it Six grams of E acetate were ground with 80 cc of 0 9 percent salt 120 cc of glass beads and about a half cc of Tween 80 for 24 hours That is somewhat over 100 000 revolutions of the flask At this time 90 percent of the particles were less than 10 microns and 30 percent were less than 5 microns That material possessed advantages which I think are greater than a water soluble preparation in that it is absorbed fairly rapidly but it is not absorbed instantly It is slowly absorbed for several hours After you stop treating a patient with 200 or 300 mg for a daily dose the effect continues for about three days This suggests that there was a reservoir with large reserves Now all of our physiological work is being done with that kind of a suspension If you dilute it down so that one cc contains 4 or 5 mg the amount in suspension actually is not great It is a very light suspension but it provides a source for some time That I think is very important

White How much E is in that original mixture?

Kendall Twenty five milligrams per cubic centimeter A very important point is that preparation of the suspension must be under sterile conditions You cannot sterilize these solutions afterward The way we do that is this the flask is sterilized with a gauze cover We put the crystals in the flask and cover them with dry ether which is an excellent bactericidal agent The ether evaporates and leaves the crystals sterile Another flask contains the 80 cc of salt 120 cc of glass beads and the Tween 80 which are also sterilized

Pincus One percent saline?

Kendall Nine tenths percent The gauze cover is removed and the saline Tween 80 and beads are added to the flask It is then stoppered with a sterile rubber stopper covered with a gauze and

rotated for 24 hours The suspension is removed from the flask with sterile 0 9 percent salt The glass beads, which are all through the suspension, are held in the flask with an appropriate glass di c fastened to a glass rod which covers the neck of the flask The beads are freed from the suspension very easily The volume is then made to 240 cc One cc contains 25 mg That is the strength we use for all the clinical work Well, I really feel as though that was an important contribution because several large pharmaceutical companies have wanted to know how it was done The difficulty, of course was the small amount of material and the impossibility to sterilize after grinding I received a letter the other day from the Micronizer Company with the information that they could produce it all right if the amount was in 100 pound lots

Thorn Isn t it important in that connection to point out that one of the traps you may fall into with compound F is that it is absorbed too rapidly and that this compound which ordinarily is somewhat more active than E becomes less active in the treatment around the clock unless the spacing is done correctly? I can never see any objection to the relatively poorly absorbed material As a matter of fact I still think there are big advantages in it because that will give you a prolonged and sustained absorption with a constant blood level A highly soluble material is nice in experimental work especially if you want to stop the material quickly, but you have to give these injections more frequently than you would with a more slowly absorbed material When you are dealing with a normal adrenal where part of your hormone is used in producing some degree of atrophy of the adrenal you have to decide whether you want the adrenal to escape between injections or not I think that is the philosophy of the dosage

Fremont Smith This discussion which I have enjoyed immensely seems to me to illustrate some of the value of this kind of meeting It would have been impossible I believe to have guessed in advance what questions would arise this morning because no one can anticipate another person s associations It has been a rewarding experience to me

REGULATION OF ADRENAL CORTICAL SECRETION

GREGORY PINCUS
Worcester Foundation for Experimental Biology and Tufts College Medical School

THIS portion of the discussion is one which should have been assigned at least dual control and I will call upon others to help out in places where I am sure I am not at all competent

In considering the subject for presentation there are several considerations that we probably ought to stress, some of which have been touched upon today

First there is one point involved in the regulation of the adrenal secretion which deserves further discussion and that is the question of the secretory products of the adrenal This is something which has always been a matter of deduction rather than observation I mean that we have deduced that the chemical substances particularly the steroids isolated from adrenal tissue are secretory products We know *that many of the products isolated may be chemical artefacts* Furthermore when I say it has been a matter of deduction from the present physiological evidence the notion is swinging to a relatively limited number of products rather than a large number of products The data on isolation of steroids supplied by Drs Kendall Reichstein Wintersteiner and their coworkers are a monumental contribution to a most difficult subject Over 20 steroids have been isolated from adrenal tissues and at present there is no adequate explanation for the great variety

In the physiological and biological studies of adrenal secretion one is confronted with problems that do not face the chemist In acute or chronic experiments in animal or man the physiological variables involved add to the difficulty of the measurements of the secretory products of the adrenal The problem is an important one If you attempt as would seem logical to isolate and determine the secretory substances coming from the adrenal glands you need such large quantities of material that for ordinary purposes in the investigation of the control of the secretions you are left way out in that field I would say I don t think it is possible even with our much improved present-day methods of determination to base all of our judgments on products from the adrenal vein We do need

reliable indices to give us measurements of secretory activity Several methods of measurements of secretory products have been reviewed and proposed First, there are methods of biological assay which have been touched on in part this morning and which in one sense are the final methods of assay that one should employ when thinking of the active hormone For example in the experiments of Martha Vogt, which will certainly come up for consideration again in this session, the use of biological assay of adrenal blood in the dog has been extremely useful The sensitivity of biological assay methods is a real advantage but the difficulty, as Dr Kendall no doubt knows is the question of specificity and of what you are measuring The discussion this morning has shown that it is difficult to be sure of the specificity of the present biological assay methods

The methods which have been used with the greatest frequency in biological assay are first, those involving glycogen deposition in the liver of the adrenalectomized animal certainly a good and valuable assay method Second the muscle work test which is a good quantitative method but which as far as I know has not been used to study secretory products directly or even somewhat indirectly Finally, there is the cold test in which the survival of the adrenalectomized animal in the cold and its protection by administered products has been measured This is the test which Vogt has used most often in her work but which also has some disadvantages

The other methods of evaluating biological activity are chemical or microchemical methods of which the principal ones may be summarized as follows 1 methods involving the study of the reducing activity of lipoidal extracts usually the neutral lipids of biological fluids Such studies have been carried on by several laboratories and moderately satisfactory methods have been evolved I believe the criticism that most of us would have and which has been stated fairly explicitly by Heard (3) in his recent review is that a degree of non specificity is involved The question of the extent to which non specific substances affect such methods is something which we in our laboratories have attempted to answer but we have, so far only a partial answer We do know that we can extract from the pool of reducing lipids in urine, for example small amounts of substances which do not have the α ketol side chain characteristic of adrenal corticoid (Romanoff, Plager and Pincus (16)) Aside from that, our information is rather limited Venning has conducted somewhat parallel experiments using ketone extractives namely Girard s reagent and she finds there is a certain moiety of non ketone reducing lipid which may or may not be steroidal

2 Another perhaps more specific, method which has been

used by a number of investigators involves the formation of formaldehyde from a neutral lipid extract In this test which should have a greater specificity, the extract is oxidized with periodic acid and the formaldehyde generated is measured Formaldehyde would be generated from two types of side chains characteristic of the adrenal corticoids either the ketol or the glycol side chain This generation of formaldehyde is a reaction which has been studied to some extent, but judging from data that we have as well as from data in the literature, much more needs to be known about this method than is known at present, particularly when applied to biological fluids and extracts

I give this very hasty review of methods of measurement because in the consideration of the regulation of adrenal secretion measurement of adrenal secretory products is essential Certainly any analysis of the pituitary adrenal mechanism must involve an understanding of adrenal secretory products if only because this mechanism involves an interaction between the two endocrines The recent clinical reports of cortisone and ACTH has stimulated interest in this mechanism and with the availability for use in the human of fairly large amounts of both substances there is accumulating and will accumulate a large mass of information on these two endocrine glands and their interactions

If one examines the pituitary adrenal relationship more closely I think that the problem of the interaction of these two glands upon each other from the secretory point of view can be subdivided for purposes of this discussion into two types of experiments 1 those involving studies *in vivo* and 2 those involving studies *in vitro* I am going to talk about the latter because we have some information on them and I will ask Dr Sayers to give us some data on the interrelationship *in vivo*

As pointed out by Dr Long in 1947 (9) the nature of the action of ACTH on the adrenal cortex is probably influenced by several factors There is also the question of what activates the pituitary and on this aspect of the problem there has recently been some interesting data Finally the negative feedback of the adrenal cortical hormones on the pituitary itself is a phenomenon which has occupied a good deal of attention

To give you as rapidly as possible the few data that we have which we consider rather direct evidence of the ACTH or pituitary adrenal relationship I will show you some data which Dr Hechter has not yet published in detail on the secretory activity of the isolated perfused gland (cf Hechter (6)) Most of these experiments have been done with the isolated beef gland, which may be

perfused *in vitro* with any of several media, although to make a long story short we find that whole blood is still the most superior of all the materials used This isolated gland can be perfu ed over a period of many hours, and during this period will produce secre tory products The gland is perfused through the adrenal artery and can be mounted in such a way that one collects only the venous effluent from the gland so one can be quite certain that the perfusion medium coming out of the adrenal vein represents fluid receiving products of the adrenal and not any adventitious material The gland may be perfused under a wide variety of pressures I cite, as an example data on a single gland in which the designated arterial pressure is the pressure registered at the cannula before there is actual entry of the fluid into the artery, so that there is probably a drop below this pressure in the arteriolar system At a pressure of 170 to 180 mm of mercury a venous output of 16 cc per minute was attained The method of measuring the corticosteroid in the venous effluent was the generation of formaldehyde after periodic acid oxidation of a neutral extract of the material and on the basis of this after some minutes of perfusion an output of 13 to 18 micro grams of corticosteroid per minute occurred Upon the injection of 1 7 mg of ACTH into the arterial portion of this system a marked change occurred within a very short period of time In a matter of 30 seconds after the injection the concentration in the venous effluent rose from 290 to 406 micrograms per 100 cc and the increased rate of output from 17 5 to 38 4 micrograms per minute In thirty seconds the output just about doubled and after another minute it increased about four fold the concentration in the venous blood increasing correspondingly

In another gland with the arterial pressure kept constant at 40 mm of Hg the production of corticosteroid was 9 4 micrograms per minute in a sample taken at 20 minutes after the initiation of per fusion when the venous outflow was 5 cc per minute Thirty seven minutes later the output had fallen to 4 3 micrograms per minute and by 98 minutes after the initiation of the perfusion (at a flow of 4 9 cc per minute) only 0 3 microgram per minute was being produced At this point the administration of 2 milligrams percent of ACTH increased the output of corticosteroid to 42 micrograms per minute 32 minutes after ACTH injection This rate continued essentially unchanged for the following two hours

These data suggest that in this isolated system ACTH directly stimulates the secretion of adrenal steroids The increase in output of formaldehydogenic lipid after ACTH has been demonstrated in a number of experiments In two experiments an apparent negative

output indicated that the concentration in the venous effluent was less than that found in the arterial blood, suggesting some absorption of the formaldehydogenic substance by the isolated gland preparation The data of some seven experiments demonstrated an invariable increase in output after ACTH Although quantitative comparisons from gland to gland cannot be safely made there was no obvious relation of the observed output of corticosteroid to the amount of ACTH perfused (it varied from 1 to 7 mgm %) perhaps because we were in each case dealing with an amount in excess of that required by an individual gland There may be some relationship of corticosteroid output to the rate of blood flow through the gland since low flow rates usually accompany lower outputs Finally the increase in output after ACTH appears to be well maintained since it is observed to be high in both the shortest (1½ hours perfusion) and the longest (4 hours of perfusion) experiments

Attempts to measure ACTH in normal blood have not been successful to date One advantage that we have in this system is that the possibility of destruction of the ACTH can be investigated

White You suspend 0 25 mg ACTH in what volume of perfusion fluid?

Pincus Our usual volume will run about 200 cc

Ralli Did you inject any other extracts from the pituitary besides ACTH?

Pincus We have injected gonadotrophic and control material which consisted essentially of non specific protein (e g gelatin) with no effect

The data also illustrate that there is an individual variation in the various adrenal glands perfused Why one gland shows only a small increase with a pressure which we consider quite adequate (80 mm of Hg) whereas a gland perfused at a blood pressure of a fourth of that (20 mm of Hg) shows a three fold greater output we don t know We are inclined to attribute it to the gland itself After all the cows taken to the slaughter house are undoubtedly subject to stress and the degree of stress varies That is, of course a factor which we would like to control but at the moment we simply take what we get As far as the sustaining of output we suspect that the amount of ACTH that we have put into the circulation is so great compared to the possible reacting tissue or the non reacting tissue that we are getting just about the maximum response we can with what is available

Sayers I think you are wise in using these large amounts because the hormone may be relatively unstable in the blood

Pincus You have evidence for that or do you just think so?

Sayers We have some evidence that the hormone is relatively unstable in blood

Pincus All these data were taken with perfusion of whole blood If we perfuse with plasma to which ACTH is added we do not get the same response We get a response of a much lower order of magnitude, and just what this means I don't know Perhaps whole blood contains a co factor for the process or simply assures oxygen adequate for the process in contrast to plasma

Long You suspect that is the case, Dr Pincus? We know that the blood flow through the gland under physiological conditions is very high and that the venous blood is practically arterial in its oxygen content Evidently a high rate of oxygen supply is essential for its activity

Pincus We have some data on that which I will mention shortly, after discussing the next set of data

All of the data previously discussed were measurements of formaldehydogenic substances Since substances other than steroids might be present in the lipid extracts which could possibly contribute to the titer we were anxious to determine the amount of biological activity in the venous effluents Dr Hechter (6) has reported four experiments in which we tested for biological activity, in perfusates obtained after ACTH administration The method used was described by Olson and his collaborators (11) and we have found it to be a fairly good method in rats *In two experiments the glycogenetic activity of approximately 1 4 mgm of formaldehydogenic steroid was equivalent to that of 1 mg of cortisone suggesting that the effluent steroid substance was either a compound less active than cortisone or that several substances were present the activity being largely concentrated in something very much like cortisone* That inactive substance may be produced is evidenced by data from two other experiments in which we could detect no glycogenetic activity in extracts containing considerable formaldehydogenic substance Thus in one experiment 400 micrograms of formaldehydogenic steroid per rat failed to produce any effect in another 90 micrograms per rat were ineffective whereas 40 micrograms of cortisone represent a minimal effective dose This may mean that certain glands obtained at slaughter are so "abused that the synthesis of active steroid is inhibited In any event it is suggested that biologically inactive material may be produced as well as indubitably active material

The next data are from some very recent work on which up to now, only two short notes have been published (*Hechter et al* (8)) We attempted to see whether the *perfusion of*

the isolated adrenal with possible precursors might give us some idea of the secretory products that could be obtained from the gland We chose as the first precursor substance 11 desoxycorticosterone By means of propylene glycol this may be put into solution well enough to be taken up by the perfusing medium Its perfusion through the isolated gland produced material which when isolated in sufficient amount was characterized chemically as corticosterone or compound B in Kendall s list This indicated the ability of the gland to take this type of precursor and not only oxygenate in the 11 position but apparently typically to produce the 11 hydroxy group

Loeb Was that on the basis of isolation?

Pincus Isolation and characterization of the product I might say within a period of two hours sufficient desoxycorticosterone was converted to this compound to allow its isolation whereas from the gland perfused without any precursor in the same period of time it was absolutely impossible to isolate any crystalline product

Kendall Circulated?

Pincus Ordinarily the blood is passed through the gland just once We have done enough experiments to be convinced that because of the order of magnitude of recovery of corticosterone that this is a conversion by the gland and nothing else For example in one experiment where we perfused 100 mg of 11 desoxycorticosterone in the course of 50 minutes we isolated 42 mg of corticosterone and that is so much beyond any possible control level that we think it is a genuine affair

Kendall Not as the acetate?

Pincus As the free steroid

Kendall You perfused the free compound?

Pincus If we perfuse the acetate what comes out is corticosterone free not acetylated

Kendall In 50 minutes?

Pincus Certainly no more than a matter of three or four hours Our standard time of perfusion will run anywhere from three to five hours

Kendall Body temperatures?

Pincus Yes

Bloch In what concentration of desoxycorticosterone?

Pincus 100 mg in about 500 cc of perfusate

Thorn No compound E after 100 mg of desoxy?

Pincus No Perhaps I had better give you a little more detail The method of extraction and of partition of these materials that

we used and which we have not published, is a chromatographic method,—we are able not only to obtain fractions which contain the corticosterone but a number of other fractions, some of which should, on the basis of model experiments segregate compounds like A or E So far we have not found either A or E in any perfusion that we have done The only compound that we have gotten out in these quantities from this DOCA perfusion is corticosterone This does not mean that other compounds may not be present since we might easily miss minor amounts We have examined a number of fractions for formaldehydogenic substance There are some which contain moderate amounts, but they are so small in ratio to the corticosterone fraction that we are certain that the major product is corticosterone

Kendall If you perfuse compound A would you expect corticosterone?

Pincus That is why I asked you for compound A I wanted to know what was going to happen

Kendall What do you think?

Pincus I have no opinion We have tried no 11 keto compounds as yet We have tried perfusion with a number of compounds but not 11 oxygenated ones That is why I was so anxious to get A We wanted to see whether the gland would reduce the ketone group and whether the gland might not introduce the 17 hydroxy function

Thorn Do you know anything about the ascorbic acid in the buffer?

Pincus Since we were anxious to have the gland working at its maximum, we added large amounts of ascorbic acid to the perfusate in some experiments But in others lack of this added ascorbic acid did not seem to alter the 11 oxygenation process

Conn This is whole blood in the perfusion medium?

Pincus No, this is with plasma You do not need whole blood You do not need the blood cells

Ralli *You said previously that when you perfused with plasma* and ACTH you did not get any increased output

Pincus The isolated gland does not give an increase in the formaldehydogenic substance when perfused with plasma and ACTH But when a precursor is put through in plasma 11 oxygenation occurs

Ralli Did you load everything with the ascorbic?

Pincus Usually, on the ground that Long and Sayers seemed to know what they were talking about when they said ascorbic acid is essential to the steroidogenic process When we add ACTH it

does not facilitate the conversion of DOCA to corticosterone

Selye You said you did everything to facilitate the work of the gland and not use the whole blood

Pincus We used both

Selye It makes no difference?

Pincus No In our more recent experiments since whole blood is hard to use as a perfusing medium for various reasons, we used the plasma

Conn What happens when you perfuse no ACTH and no pre cursor?

Pincus I gave you those figures previously There was a very small output of formaldehydogenic substance

White What happens with plasma, precursor and no ascorbic acid?

Pincus We have not done that

White Why don't you settle whether or not ascorbic acid has anything to do with the conversion?

Pincus We are going to do this Our group is limited in num bers and the things you are going to ask us to do are more than we can do in a short time

Ralli How about the amorphous compound of Dr Kendall?

Pincus I would like to try the amorphous fraction I know very little about it

Ingle Have you tried compound S?

Pincus We tried perfusing a number of other steroids to see what would happen So to summarize our information here today, I must say they were not converted in as good a yield as in the case of DOCA Every compound we have perfused is 11 hydroxy lated I will mention the steroids We have perfused with Reich stein s S and that has given us 11 hydroxycorticosterone or F in Kendall s terminology We have perfused with progesterone and obtained 11 hydroxyprogesterone

We have perfused with Δ4 androstendione and we have obtained the 11 hydroxy compound We have perfused with 17 hydroxypro gesterone and I have kept this last because it is in a way the most interesting compound from the point of view of isolable products From that perfusion we have obtained three substances of which there has been only a partial identification so I cannot be absolutely certain of all the data We have obtained fir t of all evidence of 11 hydroxylation as was true for all the other steroids we tried and in addition we obtained two other compounds one of which appears to have been reduced to a 3 hydroxy compound

Kendall Is the double bond still there?

Pincus Either a Δ4 or Δ5 Most of what I am telling you about these compounds is based on infrared analyses

Kendall Is the hydroxyl group at C 3 beta?

Pincus We know there is an acetylatable hydroxy group and since under the conditions we use neither 11 or 17 hydroxy can be acety lated, we think it is a 3 OH We know that there are certain other *positions*, just on the basis of the infrared absorption we cannot exclude all the positions So it is tentative identification of reduc tion product The reason we think it must be reduced here is that the typical absorption for α, β ketone is now gone

Kendall And it does acetylate?

Pincus It does acetylate We feel fairly confident that the 3 keto has been reduced

Kendall Does it give formaldehyde with periodic acid?

Pincus It does not give formaldehyde, but the third compound obtained does generate formaldehyde with periodic acid It does have an α, β unsaturated ketone It has been identified as Δ4, and it does have non acetylatable hydroxy groups

That is as far as we have gone in the use of precursors Again in the case of all precursors we have used under the conditions of short time perfusion, namely, no longer than five hours, *ACTH* has never assisted in the production of any new product thus far

Long You think that ACTH has no influence on 11 oxygenation?

Pincus We think that the effect of ACTH must go back of 11 *oxygenation* *The thing* we are still very *much interested in is the* introduction of the 17 hydroxy function and how that gets there Unfortunately we have no evidence We are hoping with the per fusion of compound A to get some indication of *F or E production* The effect that we did get on the 17 hydroxy progesterone side chain is most interesting since ketol generation occurred We have no indication of ketol formation after the perfusion of progesterone, but the experiment has been done only once so far As I indicated to you some glands are better than other glands *This is not* only true of the absolute *output for example in response to ACTH*, but also in the rate of conversion Some glands will convert with greater effi ciency than others Until we can repeat our experiments with really efficient glands we won t know much about the possibility of convert ing the pregnane type of side chain

White Have you had the opportunity in connection with output to investigate Martha Vogt s statement with respect to potassium and ATP?

Pincus We have done nothing about potassium We have not used ATP *per se* but we have used a muscle juice I would say that

if there is very much of a difference contributed to the 11 oxygenation of precursor we have not been able to discover it It does not mean that it does not exist but merely that we have been unable to discover it with this crude method of giving ATP We plan to do that in more detail We have also investigated the effect of epinephrine Epine phrine in no way increases the output of adrenal steroid directly

Kendall Does it slow the flow?

Pincus It does slow the flow but basing our data on the concen tration of venous effluent there is no change in the venous outflow

Long You observe a vasoconstriction in the gland

Pincus The gland slows

Long What concentrations of epinephrine will there be?

Pincus Dr Hechter has reported on an adrenal perfusion in which 10 micrograms of epinephrine added to the arterial blood led to no reduction of flow and no increase in formaldehydogenic steroid raising the amounts injected to 100 and then 1000 μg led to reduc tions in flow and corticosteroid output

Long Those were enormous concentrations compared to the amount that is usually regarded as the physiological range of epine phrine secretion

Pincus All we can say about this preparation is that ten micro grams did not decrease the flow When he went to one hundred it decreased it This effect lasted for quite a while, 42 minutes

Selye What is the normal content in the adrenal vein?

Pincus This is arterial concentration What the normal concen tration is perhaps others can answer

Gellhorn One tenth gamma per cc according to West

Long In the adrenal vein blood Dr Gellhorn 0 1 gamma per cc ?

Gellhorn West found 0 1 gamma per cc in the venous blood (ear) of the rabbit (West G B The estimation of adrenalin in normal rabbit s blood *J Physiol* 106 *426* (*1947*))

Long The effective concentration in the arterial blood can hardly be measured It is in the neighborhood of one part in twenty to thirty million

Pincus I would suggest to you that probably a good portion of this material is very rapidly oxidized in the whole blood

Thorn Unless you give epinephrine around the clock you cannot show any increase in the urinary adrenal cortical excretion products in the human

Pincus If you have constant infusion?

Thorn Constant infusion causes no increase

Pincus Is this a matter of concentration?

Thorn I think it is a matter of being up in the higher ranges constantly

Pincus The only basis on which we think this demonstrates no direct effect I might say is on the basis of the flow figures, the pro portionality of the output to flow figures That we have not touched on the more logical physiological limit is quite likely But this is as far as we can take you at the moment Without question there is much more to be done At least from our point of view the attempt was made to increase the output in order that we might be able to identify the secretory products, but epinephrine would obviously not be useful as a stimulative material

Long Those concentrations of epinephrine in the arterial blood far exceed anything which is encountered even under conditions asso ciated with the maximum output of the gland The vasoconstriction would be of the kind you would not expect from physiological amounts of epinephrine

Pincus These figures obviously indicate a large amount

Long That would damage the gland a lot

Thorn I think it is very interesting that those are right in line with our observations that epinephrine is totally incapable of increas ing 17 ketosteroids unless administered around the clock over a period of weeks

Long This is the isolated gland I think the story would be some what different if you had an intact animal

Thorn It isn't any different

Pincus I think Vogt s data on this sort of thing are not data They are just statements At least I have not seen the data What she says is that she has gotten some indication of increased output with epinephrine

Gellhorn Does she not also state that the stimulation of the splanchnics likewise increases the output of adrenocortical hormones?

Pincus If you consider the so called secondary effect of ACTH output on the adrenal—certainly not shown in the hypophysectomized animal—you don t think that is so?

Long That is correct You remember in her first paper she had one experiment with a freshly decapitated dog Of course the blood pressure would be low in such a case As I understand it she claimed in that preparation that epinephrine still increased the output of cortical hormone as measured by her method I believe in more recent experiments she was unable to obtain any increase with epinephrine in the absence of the pituitary

Loeb I think that is quite right—at least when she was with us she was convinced it was not a direct effect

Thorn Interrupted epinephrine injected every four or six hours is a different story The vasomotor effect lasts only for a few minutes after injection and then you have the effect of ACTH stimulation of the anterior pituitary left I thought that continuous round the clock infusion would be the best way to stimulate the gland, but we have never been able to get maximal stimulation that way

Selye In the hypophysectomized rat the administration of adrenalin three times a day does not cause any detectable adrenal change We used adrenalin as one of our standard alarming agents in our work on the general adaptation syndrome and found that hypophysectomy prevents its effect (as that of any stressor) on the adrenal

Long I recall from Martha Vogt s paper where she injected epinephrine for ten days and found it produced adrenal enlargement in the normal animal but not in the hypophysectomized

Pincus The deduction from these data is that a direct effect of epinephrine is not proven In fact, it may inhibit steroid output I think that is as far as we can go I agree that more might be done with perhaps arterenol

Ralli What do you think would happen if you left out the ascorbic acid from your perfusion medium?

Pincus As stated previously 11 oxygenation is not interfered with when ascorbic acid is not added to the perfusing medium What the effect would be in longer term experiments I cannot say Let me be explicit What comes out in the case of desoxycorticosterone transformation is corticosterone as the free compound We don t do any hydrolysis to free it It is directly extractable from the perfusate, so there seems to be no indication of conjugation of these materials with anything coming out directly from the adrenal As already mentioned the acetates are deacetylated

Kendall The red cells contain an enzyme which will do that, isn t that so?

Pincus We don t know whether the red cells or the gland itself is responsible

Loeb Did Dr Pincus use plasma not whole blood?

Pincus That is right

Bloch It might be lipase that would do that

Pincus It might very well What we should have done was simply to incubate acetate with plasma or blood to see if the material was changed We have not done that but that experiment is sure to be done before very long and we will have the answer

Ralli Have you tried cholesterol?

Pincus Cholesterol is present in considerable amount in the

perfusing medium, in the fractions we have explored there are no evidences of 11 oxycholesterol

Thorn What happens when you add a little adrenal brei to the progesterone?

Pincus We have just started some experiments on adrenal slices, adrenal brei and adrenal extracts and so far the conversion is much less than in the perfused organ

Thorn You do get some?

Pincus We get some We have only used desoxycorticosterone and it appears to be converted to corticosterone

Kendall How about recirculation?

Pincus Do you want to hear about that? That is very interesting When we repeat the circulation of the perfusate there is a further conversion of the remaining desoxycorticosterone, apparently at about the same rate, but a certain proportion also disappears Eventually all of the DOCA will be used up, but the amount appearing as corticosterone varies with the gland used, since some glands are more efficient than others

Kendall If you circulate the corticosterone?

Pincus We don t know That is an experiment we are trying, and also A

Selye If I remember correctly what you said, you obtained about 50 percent of the DOCA in the form of corticosterone after a single perfusion?

Pincus You can in a good gland

Selye All that happens in a good perfusion is that the original corticoid is destroyed?

Pincus There is a big distinction between glands Some glands give only poor conversion, as poor as 3 percent and others give good conversion as good as 50 percent In the poor glands or good glands it does not make much difference, you cannot account for all the material even in a single passage some material has disappeared into unknown metabolites

Conn Does ACTH convert a poor gland into a good gland in this respect?

Pincus ACTH will take any poor gland and cause it to put out formaldehydogenic steroids

Conn Is the poor gland treated with ACTH better able to bring about the conversion from the desoxy compound?

Pincus We have not done that experiment on the same gland In a way I should apologize for talking as much as I have about these experiments It is obvious from the answers I have given

they are rather incomplete There are at least two things we can say One is that the gland is capable very obviously of introducing 11 hydroxy groups and also apparently of converting the progesterone type of side chain at least in 17 hydroxylated compounds to the E or F like side chains What other things the gland can do is still a mystery Furthermore it should be emphasized that the isolated gland is no longer "normal " so it may only partially perform the total job of the gland *in vivo*

This attempt to get some idea of the secretory products of the gland by the use of the isolated preparation has been applied to other endocrine glands We have attempted, for example, similar experiments with ovaries studying the effects of gonadotrophins on ovaries, and there the increase in estrogen output is very small compared to the large increase of corticoid output which you get from the adrenal There is an increase but it is really quite limited

Selye Are those ovaries in the follicular or corpus luteum phase?

Pincus We have tried it in every phase We have tried rabbit ovaries and the rabbit ovary is pretty small as an ovary Maybe we should use a larger ovary We have tried the cow ovary to a limited extent and I must say we are not very much encouraged We have had cow ovaries in the follicular and the luteal phases There has been an increase in estrogen after gonadotrophin but it is small We have also begun some experiments on possible precursors and so far I have nothing to report Our clues are thus far rather meager If one tries to get some idea of this secretory capacity in other types of preparation one obviously is faced with much greater difficulty For that reason I think that one has to be very careful about the data that are available thus far

I suppose in a way our best data on the effects of ACTH on adrenal secretion are had in man where the administration of ACTH in good amounts within recent time has provided some opportunity for the study of the products which appear in the urine As Dr Bloch pointed out this may neglect an important avenue of excretion but I know of no data along those lines In our laboratories we have confined our attention to the measurement of classes of urinary compounds rather than isolation experiments to date Isolation experiments have been done by Dr Mason and also by Dr Dobriner and his group I cannot speak in detail about their data Dr Kendall knows Dr Mason s data much better than I do I have seen a tabulation of Dobriner s data and it is quite obvious that two situations exist Judging on the basis of his data with low doses of ACTH the output of urinary steroids is merely increased quantitatively After high doses of ACTH there is not only a quantitative

increase in the known characteristic steroids, but also the appearance of a new group of steroids which has not been completely characterized as yet What the significance of this is, is hard to assess I don t know particularly why a small dose of ACTH should have merely a quantitative effect whereas a large dose should have a qualitative effect I suppose it is partly due to the efficiency with which one can detect the new metabolites involved In our own experience and that of many other people, the increase in 17 ketosteroids in man is regular and I think unquestioned after the administration of ACTH What the 17 ketosteroids represent is a problem which has been discussed many times I think we are going to have some answers now that typical corticosteroids will be available in sufficient amounts for metabolism studies

As far as the reducing lipids or the corticoid substances are concerned, measurements have been made by every method of assay that I have mentioned except the glycogenic steroid output there the increase is also undoubted after ACTH We have attempted to fractionate the urinary corticoids in order to get some idea of what the products might be This is something which I think Dr Mason probably has done much better than we have and our results are in essential agreement

The one substance which we are certain is increased in output after ACTH is F Everything else is still a matter of question The fractionation of urinary substances is still at least in our hands something which is in large measure a technical chemical problem The chromatographic separation that we have employed seems at the moment quite satisfactory, but we are still working on it We *have developed methods for the measurement of these substances* which we expect to publish soon indirect methods, colorimetric reactions chiefly Perhaps with the use of fairly specific color reactions or something very similar to that we may get a better clue as to the type of substance involved

One feature of our studies of colorimetric reactions has baffled us, and Dr Kendall might be interested in this The reagent which we are using is antimony trichloride, which is a favorite of mine because it is a very reactive substance with steroids and you get, for example, with desoxycorticosterone a remarkably sensitive reaction in which a bluish compound with red fluorescence is produced You get a fluorescence reaction with F You get fluorescence with corticosterone although the reaction product with F, the colored substance, is somewhat different It is not blue It seems to be a much paler color As soon as the 11 keto group is introduced you get no color, *no fluorescence* of any kind In other words if you

take compound A and tried it with antimony trichloride, you get no reaction If you take E you get no reaction, and we have had similar experience with one other 11 keto compound

Kendall Anhydrous medium?

Pincus Anhydrous medium, but if there is a hydroxy group present or no oxygen function at all, we get the fluorescence reaction Why the 11 keto group should interfere absolutely quench that reaction, is remarkable It is quenched particularly as the fluorescence is concerned and that is very mysterious

Kendall I think there may be an explanation If there is a hydroxy group dehydration will occur It may be the water which affects the color

There is another color reaction which is mysterious Desoxycorticosterone with sulphuric acid if kept in a vacuum desiccator does not develop any color but if you allow a little oxygen to enter color appears If you allow water vapor to enter a beautiful blue color develops according to how much oxygen and how much water there is

Pincus How do you explain it in the case of 11 desoxy and S which also will give this color? In other words where there is no 11 oxygen function at all

Kendall I cannot explain that

Pincus I cannot explain that either I want very much to call on Dr Sayers to discuss some pituitary adrenal relationships and then to call for general discussion Is that agreeable with you Dr Long?

Long Certainly!

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DISCUSSION

Sayers We have been using the adrenal ascorbic acid depletion technique as a method of measuring rate of discharge of adrenocorticotrophic hormone from the anterior pituitary When an animal is exposed to stress there is an immediate discharge of adrenocorticotrophic hormone from the anterior pituitary which induces a reduction in the adrenal ascorbic acid The reduction will occur within a few minutes after exposure of the animal to stress or after the administration of adrenocorticotrophic hormone We have selected

a period of one hour after the application of the stress as a convenient time at which to measure hormone discharge A neural mechanism could be involved in regulating pituitary adrenocorticotrophic hormone activity We found that section of the infundibulum had no effect upon the discharge of adrenocorticotrophic hormone from the anterior pituitary which normally occurs when an animal is exposed to stress

As you know G W Harris in England has demonstrated that following section of the stalk there occurs regeneration of what he calls neurovascular links between the tuber cinereum and the adeno hypophysis (Harris G W Neural control of the pituitary gland *Physiol Rev* 28 No 2 *139* (*1948*)) The experiments on neural mechanisms were extended to include animals in which adenohypophyseal tissue was transplanted to the anterior chamber of the eye and to the spleen The number of takes were quite small It is a difficult technique and Dr Chi Ping Cheng who conducted these experiments must be congratulated for his patience and skill Thirteen animals had viable tissue grafts as determined by histological study In 4 of these animals the rate of discharge of adrenocorticotrophic hormone in response to histamine stress was equal to that of animals with intact stalks In 7 of the animals there was an intermediate type of response a response between that of a normal animal and that of a hypophysectomized animal In 2 of the animals with viable grafts—viable according to the histologist—the response was equivocal and I would not want to say that there was a positive response The experiments demonstrate that a direct neural path between the hypothalamus and the adenohypophysis is not essential for the discharge of ACTH during stress Since we could remove the adenophypophysis from its normal site and still get discharge of ACTH in response to stress a neurovascular link as described by Harris does not appear to be an essential link in the mechanism

We have also studied the matter of hormonal regulation of pituitary adrenocorticotrophic activity that is the influence of the blood titer of cortical hormones on discharge of ACTH The first conclusive experiment was carried out by Dr Ingle who demonstrated in animals forced to exercise for 12 hours that there was a measurable hypertrophy of the adrenal If these same animals were given appropriate doses of adrenal cortical extract during this period of time there was no hypertrophy of the adrenals This suggests that the hypertrophy was connected with a need of the organism for additional quantities of cortical hormone during exercise (Ingle D J The time for the occurrence of cortico adrenal hypertrophy in rat during continued work *Am J Physiol* 124 *627* (*1938*))

With the development of the adrenal ascorbic acid depletion technique we were able to extend the experiments of Dr Ingle to more acute situations—periods of an hour following stress We were able to demonstrate that pre treatment of an animal with cortical hormones can prevent the discharge of ACTH which normally occurs A variety of stresses were studied—histamine, killed typhoid epine phrine, heat and cold—and in all five instances discharge of ACTH could be prevented

I might say that when you work with an animal that is exposed to a rather severe stress, for example if you employ doses of hista mine which are toxic enough to induce collapse, then under those circumstances we have not been able to completely inhibit the dis charge of the hormone from the anterior pituitary Pituitary physi ology is complex, and one can think of a number of possibilities which might explain these results In the first place we may not have used large enough quantities of hormone The possibility also exists that the use of histamine in doses which produce cardiovascular collapse results in anoxia of the adenohypophysis and liberation of ACTH due to increased permeability of cell membranes to the hormone

Selye How much and what kind of cortical preparation did you use?

Sayers We were using 17 hydroxycorticosterone at a dose of one milligram infused over a period of one hour I think the experiment should be repeated with larger doses of the hormone

Selye What would you consider a large dose?

Sayers That is hard to say If I were going to do it again I would try ten milligrams Incidentally there are the factors of absorption and destruction of these hormones Dr Dougherty has recently discovered for instance that if one milligram of cortisone is given one hour before an animal is challenged with antigen the hormone offers some protection whereas if given in divided doses (2 milligrams total) at 2 hours and at ten minutes before the antigen, cortisone will give complete protection

Loeb Will you say that again?

Sayers Dr Dougherty has shown that anaphylactic shock can be induced in adrenalectomized mice In the intact animal anaphy lactic shock does not occur even when the animal is challenged with one cc of horse serum In the case of the adrenalectomized mouse 5/10 000 cc of horse serum will produce anaphylactic shock and death If you give the cortisone one hour before the animal is challenged slight protection occurs if divided doses are given at

2 hours and at 10 minutes before the antigen complete protection occurs

Thorn How is it given?

Sayers Injected intraperitoneally

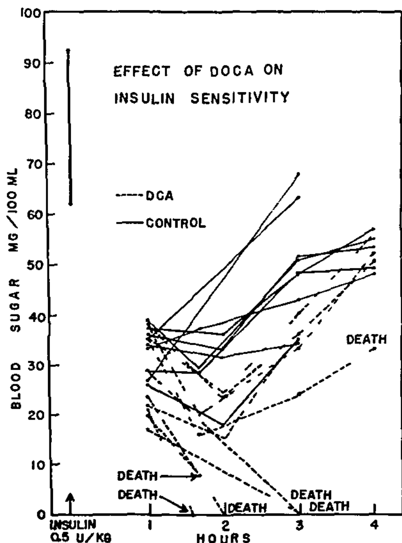


FIGURE 3 The effect of DOCA admini tration in demedullated rats upon insulin sensitivity Regular insulin was injected intraperitoneally in a dose of 0 5 unit per kg at zero time The ertical line at zero time represents the range in fa teng blood sug r of all rats in series I

Selye Crystalline?

Sayers Aqueous So I say there are problems here of fate and excretion of hormones which complicate our story I believe with our present knowledge of rate of destruction of cortical hormone inadequate as it is, we could perhaps go back and study the problems of pituitary regulation a little more intelligently than we did a few years ago

We have examined a variety of steroids with regard to their pituitary inhibitory potency and in particular we examined desoxycorticosterone and 17 hydroxycorticosterone 17 Hydroxycorticosterone was more potent than desoxycorticosterone but the very fact that desoxycorticosterone did have an inhibitory effect has important implications It was predicted that desoxycorticosterone, if given in large enough doses, would induce a state of adrenal cortical insufficiency as far as the production of compounds like 17 hydroxycorticosterone is concerned We have some indirect evidences in this connection which I would like to show you

Figure 3 is concerned with insulin sensitivity All the animals were adrenal demedullated Half the animals were given DOCA, the others were untreated The animals given DOCA were hypersensitive to insulin a metabolic disturbance characteristic of a deficiency of 11, 17 oxysteroids of the adrenal cortex

It has been shown in our laboratory by Woodbury and coworkers that the administration of desoxycorticosterone increases the electroshock threshold of rats that is decreases the excitability of the central nervous system Figure 4 shows the effect of DOCA and ACTH

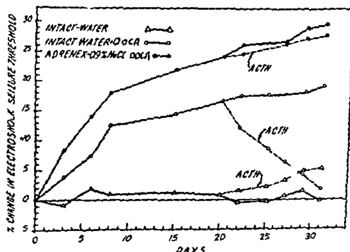


FIGURE 4

and combinations of DOCA and ACTH on electroshock threshold of rats Following implantation of desoxycorticosterone (six 15 mg pellets) the electroshock threshold steadily rose ACTH had no effect on the action of DOCA in adrenalectomized rats On the other hand in intact animals adrenocorticotrophic hormone brought the electroshock threshold down to normal ACTH without DOCA did not have an appreciable effect Here is another bit of indirect evidence which indicates that stimulation of the animal s adrenal with ACTH counteracts the effect of desoxycorticosteron

Pincus Stimulation with ACTH?

Sayers Yes stimulation with ACTH Table I presents plasma electrolytes in rats treated with DOCA and ACTH The normal level for plasma sodium is about 142 5 milliequivalents per liter Animals given DOCA have plasma sodium levels over 150 milliequivalents per liter

Pincus What dose?

Sayers The dose was six 15 mg pellets and it was allowed to be absorbed for a period of 30 days When ACTH is given simultaneously with DOCA plasma sodium level is normal ACTH alone had little effect in the doses employed The changes in sodium parallel the changes which I just described as occurring in the electroshock threshold ACTH reduces plasma potassium as does DOCA but DOCA and ACTH are not synergistic as far as the reduction of plasma potassium is concerned

I don t want to go into this matter of the toxicity of desoxycorticosterone in too much detail but let me describe briefly some experiments employing unilateral nephrectomized rats given 0 9% sodium chloride to drink and implanted with DOCA pellets These experiments have been conducted by Dr Dixon Woodbury and Dr Charles A Rosenberg Besides the group of animals given DOCA alone we had a group of animals given DOCA plus ACTH We examined the heart, pancreas kidneys and joint There were no lesions in the joints in any of the animals In the heart interstitial myocarditis and in the pancreas periarteritis nodosa developed In the kidney we did not find very striking effects but in certain of the animals changes occurred in the glomeruli (focal glomerulitis) We have graded the lesions in these animals (Table II) and in Experiment No 1 you can see that in the animals given DOCA and ACTH simultaneously a certain amount of pathology developed With DOCA alone the pathology was definitely greater The untreated animals had no lesions In Experiment 2 DOCA alone did not induce as striking changes as in Experiment 1 The combination of DOCA and ACTH was certainly no worse than DOCA alone if anything less

TABLE I
PLASMA ELECTROLYTES PER KG OF PLASMA FOR RATS

Electrolytes	Na		K		Cl		Mg		Ca	
Treatment	mEq	N*	mEq	N*	mEq	N*	mEq	N*	mEq	N*
Untreated	142 6 ± 0 8	8	4 70 ± 0 16	8	105 0 ± 0 9	6	2 3 ± 0 04	10	5 50 ± 0 09	13
DOCA	151 2 ± 1 2 (0 001)	8	3 32 ± 0 07 (0 001)	8	96 3 ± 1 0 (0 001)	5	1 78 ± 0 07 (0 001)	8	5 52 ± 0 05 (0 6)	8
ACTH	143 9 ± 0 4 (0 2)	8	4 64 ± 0 18 (0 8)	8	—		—		—	
DOCA ACTH	143 8 ± 0 6 (0 2)	8	3 57 ± 0 12 (0 001)	8	101 3 ± 1 2 (0 2)	7	2 47 ± 0 11 (0 1)	8	—	
Adrenex DOCA	148 9 ± 0 5 (0 001)	7	2 95 ± 0 09 (0 001)	7	—		—		—	
Adrenex DOCA ACTH	148 5 ± 0 3 (0 001)	9	2 91 ± 0 06 (0 001)	9	—		—		—	

*N = number of rats

Note Figures in parenthe es are P values of differences from untreated rats

TABLE II
EFFECT OF DOCA AND DOCA PLUS ACTH ON THE DEVELOPMENT OF PATHOLOGICAL CHANGES IN RATS

Experiment	Treatment	Heart	Pancreas	Kidney
1	DOCA ACTH	0	±	0
		±	±	0
		±	++	+
	DOCA	++	+++	++
		+++	+++	+
		+++	+++	+
		+	++	0
	Untreated	0	0	0
		0	0	0
		0	0	0
		0	0	0
2	DOCA ACTH	±	+	0
		±	+	0
		±	0	0
		0	0	0
	DOCA	±	+	0
		±	0	0
		±	0	0
		±	+++	+
	Untreated	0	0	0
		0	0	0
		0	0	0
		0	0	0

severe Unfortunately the development of lesions by DOCA is not as reproducible as we would like it to be However the purified ACTH we employed did not aggravate the lesions which are induced by the DOCA ACTH actually seemed to counteract the toxic effects of DOCA

Selye Were all these animals unilaterally nephrectomized and kept on sodium chloride?

Sayers Yes The controls were unilaterally nephrectomized and given 0 9% sodium chloride to drink

Selye And the doses of DOCA and ACTH respectively were?

Sayers DOCA in this case we implanted six 15 mg pellets and the dose of ACTH was 3 mg per day given in three divided doses

Selye How long did the experiment last?

Sayers The experiment lasted thirty days

Selye How heavy were the animals?

Sayers The weight of the animals was 200 grams They were males

Selye I may say here that the females are usually a little more sensitive and the best results are obtained in rats of about 60 to 80 grams

Sayers I realize we could discuss this aspect of the problem at some length However, time does not permit It is an important aspect of the problem of regulation of the anterior pituitary The three pieces of indirect evidence,—first, insulin hypersensitivity after DOCA, second, counteraction of DOCA effect on electroshock threshold and plasma sodium by ACTH and ACE, and third, suggested evidence that ACTH will counteract the pathology induced by DOCA—support the thesis that DOCA inhibits the adrenocorticotrophic activity of the adenohypophysis and in so doing induces a state of hormone imbalance characterized by an excess of DOCA (administered) and a deficiency of endogenous 11 17 oxysteroids

The epinephrine story I think requires a little bit of elaboration As I said before we found that epinephrine would stimulate the adrenal cortex as shown by the reduction which it produced in adrenal ascorbic acid However we could inhibit this adrenal ascorbic acid depleting effect by the infusion of cortical steroids We interpret this to mean that epinephrine in its actions is discharging adrenocorticotrophic hormone and acts indirectly on the anterior pituitary and that epinephrine very likely acts like other non specific stresses in bringing about a greater utilization of corticosteroids in the peripheral tissues and by that mechanism brings about the discharge of ACTH

Adrenocorticotrophic hormone is released from the adenohypophysis which in turn stimulates the adrenal cortex to secrete cortical hormone the cortical hormone is 'utilized by the tissues of the organism Utilized is used with reservations since we have meager information about the intercellular processes concerned with degradation and function activity of cortical hormone A certain titer of cortical hormone is maintained in the venous blood and this titer in some way or other regulates the discharge of adrenocorticotrophic hormone from the adenohypophysis Under optimal conditions a small quantity of cortical hormone is produced, a small concentration exists in arterial blood and this is sufficient to maintain eucorti

cism for the peripheral tis ue cells require relatively small amounts of the hormone However, in stress there is a much greater need for the hormone and ACTH is discharged in greater than normal quantities in order to increase production of cortical hormone by the adrenal cortex Naturally the concentration of cortical hormone in arterial blood must be elevated to meet the increased needs of the peripheral tissues venous blood on the other hand may if anything be slightly less or equal to that which occurs under optimal conditions The proof of the concept requires an experimental approach which is technically difficult—analysis of both arterial and venous blood for the cortical hormone We may not be too far off from such an analysis It would certainly help settle these speculative points Let me emphasize that the pituitary adrenal system cannot be perfect in regulation in the sense that it maintains a very fixed level of corticosteroids in the blood On the contrary it must allow a certain fluctuation in titer to take place in the venous blood If we may use an analogy it is something like the maintenance of the level of blood sugar where a relatively fixed level of blood sugar is maintained but which does vary within certain limits even in an individual with normal regulatory mechanisms

Now going back to the epinephrine it seems to me that if epinephrine has some direct action upon the hypothalamus or anterior pituitary to bring about the discharge of ACTH administration of epinephrine should induce hypercorticism In other words a mechanism of this sort a central driving mechanism would not pay too much attention to what is going on in the periphery

Long You still have got self limitation if the effect of your hypothesis is correct

Sayers I understand epinephrine has a very slight therapeutic effect in the collagen diseases but certainly it does not approach the therapeutic action of adrenocorticotrophic hormone or cortisone Apparently epinephrine is exciting the pituitary adrenal system but only to the extent that cortical hormone production is increased in order to meet the additional requirements induced by epinephrine in the periphery Undoubtedly some benefit may be expected with epinephrine since the elevation of hormone titer in arterial blood must give the various tissues of the organism in particular connective tissue in collagen diseases a slightly elevated titer of cortical hormone

Long The effect is bound to be self limited from your own argument and from your experiments

Sayers That is true

Long You are dealing with a homeostatic system

Thorn We have shown by methods of direct blood examination for steroid and by the eosinophilic response, that after epinephrine you get a high level of 11, 17 oxysteroids This might block further ACTH discharge

Sayers In arterial blood?

Thorn In the venous blood

Sayers How did you assay it for the corticoids?

Thorn By an animal assay using the fall in eosinophils in the adrenalectomized, gonadectomized mouse

In addition we found that after two weeks of epinephrine injections, given every 6 hours, the 17 ketosteroid excretion in the urine doubled

Sayers What escapes into urine is, of course, dependent chiefly on arterial titers Admittedly a very small quantity of cortical steroids escapes into the urine

Thorn If epinephrine is hitting the higher centers, a rise in blood steroids might make epinephrine increasingly effective and yet these same steroids may also lead to anterior pituitary depression

Sayers That is what does happen

Conn I cannot quite understand how if the source of adrenal corticoids is always the venous system how the arterial concentration can ever be higher than the venous concentration The source is from the venous side isn t it?

Sayers The adrenal blood dumps directly into the vena cava, then to the large vessels

Thorn The arterial titer still has to be lower

Conn Yes, the arterial cannot be higher

Sayers Adrenal vein blood must have a higher titer than arterial, but arterial must be higher than peripheral venous blood Dr Vogt in her experiments was able to detect cortical steroids in adrenal vein blood but she was never able to detect it out in the periphery in any large vessel Here is a technical problem Methods of assay are not sensitive enough to determine A V differences in cortical hormone titer The concept which I have presented is a working hypothesis, experimental test will determine its value

Long This effect of epinephrine is of course an extremely interesting one from our experience with it It is not very simple to analyze but you have certainly two possibilities for this effect of epinephrine due to the nature of the hormone You have the possibility of a very rapid action In addition the metabolic effect of epinephrine is characterized by increased metabolic rates which may rise 40 50 percent That does not take place in seconds but

in minutes It is a slow effect What I find difficult to understand is whether the whole explanation of the effect of epinephrine is due to the metabolic effect The stimulation of sensory nerve, whether it is just painful injection under the skin is followed in a matter of minutes by a discharge of the adrenal ascorbic acid Indeed, painful stimulation of any nerve which to my mind could not possibly increase the metabolic rates of tissue in that period of time can certainly give rise to numerous nerve impulses in the nervous system this causes epinephrine discharge which is immediately followed by discharge from the adrenal cortex as shown by the ascorbic acid level

Gellhorn Isn t it correct for *in vitro* experiments that if you add to tissues very small concentration of adrenalin you find increased metabolism as soon as you can measure it? That is an old experience of Ahlgren (Ahlgren G *Skand Arch Physiol* 47, *275* (*1925*)) and others (Abderhalden E and Gellhorn E *Arch ges Physiol* 212, *523* (*1926*))

Long Isolated tissues?

Gellhorn Yes

Long You may be correct Dr Gellhorn

Pincus If it could be demonstrated that anything other than epinephrine has the effect the specificity would disappear We have injected sugar and found the drop in adrenal ascorbic acid as fast as we could take the adrenal out

Long If you expose a vein or cut down on a vein in an animal or stick a needle into it you have done a lot of other things besides injecting sugar These are sufficient to promote epinephrine discharge in themselves

Pincus In other words the epinephrine discharge must occur presumably hypothalamically Do you think it could be adrenomedullary and act that way?

Long The pathway for reflex secretion of epinephrine is up to the pontine or thalamic area and down over the automatic centers

Gellhorn I got interested in the mechanism involving adrenalin on the basis of three facts First it was reported by Long that adrenalin depletes the ascorbic acid content of the adrenal gland Secondly it was shown by Dougherty and White that the injection of the adrenocorticotrophic hormone produces lymphopenia and third it was shown by Pincus and collaborators that conditions of stress result in lymphopenia If that is so it was to be expected that since the stress condition involves a secretion of adrenalin through the sympathetic mechanism that adrenalin would produce a lympho

penia We carried out such experiments and found that very small amounts of adrenalin as low as 05 gamma adrenalin per 100 gram *weight of rat actually produced lymphopenia* (Gellhorn, E and Frank, S *Proc Soc Exp Biol & Med 69, 426* (*1948*)) Then we began to study conditions involving sympatheticoadrenal discharge using lymphopenia as an indicator We studied the effect of hemorrhage (Gellhorn, E and Frank, S *Proc Soc Exp Biol & Med 71 112* (*1949*) and unpublished work), hypoglycemia convulsions and cold, and in all our experiments we carried out comparative studies on normal adrenodemedullated and adrenalectomized animals It was shown before by Pincus and collaborators that various conditions of stress produced lymphopenia in normal animals but not in adrenalectomized animals The fact that we added was that the lymphopenia did not occur under conditions of stress in adrenodemedullated animals From that we concluded that the sympathetic discharge lead to the activation of the adrenal medulla which in turn, possibly through the mechanisms which Dr Sayers has outlined, *caused an increase in the secretion of adrenal cortical hormones* and thereby lymphopenia

Now as to the central mechanisms involved I have this result to report we studied the effects of cold on lymphopenia (unpublished data) and found again in line with the previous experiment, that normal animals subjected to cold show a very marked lymphopenia and that adrenodemedullated animals under the same conditions fail to show lymphopenia and behave exactly as adrenalectomized animals do We then subjected these animals to pentothal anesthesia with the following results in four groups of experiments unanesthetized animals subjected to cold show lymphopenia as you would expect pentothal animals subjected to cold do not show lymphopenia They behave just like pentothal animals at room temperature If pentothal animals at room temperature are given adrenalin they show marked lymphopenia as a matter of fact they show a greater lymphopenia than the unanesthetized animals subjected to cold We have extensive material on these experiments because we carried out the cold experiments not only in animals which were subjected to ice water, but also another series in which the rats were put in the refrigerator for one hour, and the results in both groups checked very well It is rather peculiar that the anesthesia with pentothal prevents the occurrence of lymphopenia In my opinion it can only mean that the sympatheticoadrenal discharge does not take place under these conditions because the same animals injected with adrenalin show the same effect

Fremont Smith The pentothal animal injected with adrenalin showed the effect?

Gellhorn Showed a greater effect than there ever was produced by cold even in unanesthetized animals Apparently the amount of adrenalin liberated is not as great as was injected experimentally

In the experiments to which Dr Sayers referred his recently published work on histamine I noticed with great interest that he found if I am correct that histamine produced a depletion of adrenal ascorbic acid in pentothal anesthetized animals

Sayers We used sodium pentobarbital

Gellhorn There is no fundamental difference In other words the anesthetized animals did give a clear evidence of adrenocortical discharge in spite of barbiturate anesthesia I think that is interesting because trusting Dr Sayers experiments as much as I trust mine I would say that the level of the central nervous system involved in the two conditions must be different because the barbiturate narcosis can easily involve the hypothalamus for instance, but it is very unlikely that it would involve the medulla or the spinal cord If you inject histamine and you get a very marked fall in blood pressure I believe that the sympatheticoadrenal discharge which is involved in all of these experiments, would still be intact because it is mediated through the carotid sinus peripherally and the medulla oblongata centrally and would be present even in the decerebrate animals The cold experiment probably involves the hypothalamus I do not want to be misunderstood in the interpretation of this experiment It is not to be inferred from it that adrenalin involves a direct stimulation of the hypothalamus of the hypophysis I criticized Dr Long s interpretation as stated in his paper that the effect of adrenalin to produce a depletion of adrenal ascorbic acid is not obtained in the hypophysectomized animal in spite of the fact that an implant of the hypophyseal gland was given I believe that adrenalin would not act on the hypophysis for the simple reason that all adrenalin could do would be to bring about a vasoconstriction i e a vasomotor change There is considerable evidence in the literature that vasomotor changes do not bring about changes in the secretion of the glands of internal secretion and in fact positive evidence on this point is far from convincing

Long Those implants were not viable though Dr Gellhorn They were simply given as a convenient way of maintaining the adrenal The intent even if I did not make it clear in the paper was that these were simply implants to give the animals a supply of corticotrophic hormone each day It may be misleading

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Gellhorn I see

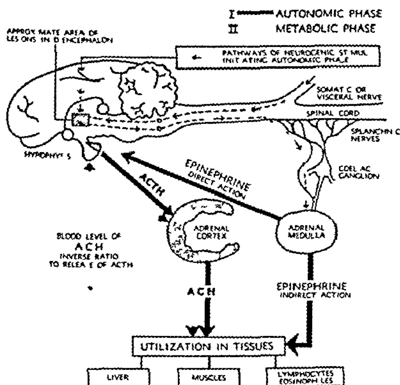
Long The same condition obtained in other experiments in that paper In still other experiments, we gave ACTH for the same purpose We did this because in discussion with Martha Vogt she had said that possibly the failure to evoke response with epinephrine in an animal after hypophysectomy was due to the atrophy of the adrenal cortex This is a good point that the gland was so reduced in activity it had to be built up again before it would respond So that is why in the experiments you were mentioning we had maintained the adrenal with ACTH or more conveniently by simply implanting rat pituitary The intent was not to imply those were viable transplants in the sense Dr Sayers was talking about

Gellhorn I would make a guess, even in the case where you have viable implants, the reactivity of these implants to a lowering in the adrenal cortical level in the tissues is very much diminished, if it is present at all

Now as far as the adrenalin is concerned my idea is simply this whenever we have a condition of stress we have a sympatheticoadrenal discharge which is responsible—I am inclined to adhere to Dr Sayers' interpretation—for an increased output of adrenocortical hormones If the sympatheticoadrenal discharge originates from the higher centers of the brain such as the hypothalamus narcosis may eliminate it If it starts from lower centers, narcosis would have very little effect The separation of the hypothalamus from the hypophysis would according to this interpretation be without any effect on the reactions which are under consideration, and I am very glad to see that in Sayers experiment no such effect has been found

From the point of view of neurophysiology I believe that the data mentioned here are of considerable interest for this reason we have been used to speaking of the sympatheticoadrenal system and we know that whenever we have a sympathetic discharge we have at least potentially an adrenalin discharge as well The experiments reported here lead one to believe that under such conditions the changes in the organism are far greater than was assumed heretofore Apparently sympatheticoadrenal discharges are followed by increased adrenocortical discharges which are due to an involvement of the pituitary In other words the autonomic nervous system is really an activator of the endocrine system and a good many changes which occur as the result of the sympatheticoadrenal discharges may be attributed to the e endocrine discharges I believe this means a change in our outlook concerning the importance of the sympatheticoadrenal discharge

A final remark. I said in one of my papers that since I didn't believe adrenalin would work on the hypophysis, it would work on the central mechanism on the hypothalamus (Gellhorn, E. and Frank, S. *Proc. Soc. Exp. Biol. & Med.* 70, 107 (1949).) and I forgot that I myself had shown a number of years ago in collaboration with Darrow (Darrow, C. W. and Gellhorn, E. *Am. J. Physiol.* 127, 243 (1939) and *Proc. Soc. Exp. Biol. & Med.* 40, 487 (1939).) that the excitability of the hypothalamus is actually diminished under these conditions, i.e., if small amounts of adrenalin are used. Adrenalin represents really a homeostatic mechanism as far as the sympatheticoadrenal discharge is concerned. Whenever we have a condition leading to an increased sympatheticoadrenal discharge, the adrenalin tends to diminish the excitability of the center which is responsible for the sympatheticoadrenal discharge.



PROPOSED MECHANISM of CONTROL of the SECRETION of ADRENAL CORTICAL HORMONES

FIGURE 5

Long I would just like to show Figure 5, which will perhaps help the discussion of what I am going to say This shows the spinal cord, the pituitary and the hypothalamus As I said, we have been very interested in the effect of epinephrine in promoting discharge of the adrenal cortical hormones We asked ourselves whether the effect could be directly on the pituitary or possibly the hypothalamic area, or whether there are any connections passing down from the hypothalamus or adjacent structures into the adenohypophysis To complete this pathway here we have the splanchnic nerves and the various sympathetic centers in the cord and the hypothalamus that are related to epinephrine secretion We know from Cannon's work and others that the impulses leading to the secretion of epinephrine enter the cord at different levels and pass upward to the pons and hypothalamus and back down over the splanchnics to cause a reflex secretion of epinephrine As far as I know, everybody is agreed on this point This a long pathway, up to the corpora quadrigemina and into the hypothalamic area In order to get some further idea on this mechanism, we have prepared a whole series of rats in which the cord was transsected about the level of D3 and D4 In these animals the long pathway for epinephrine secretion is interrupted Stimulation below the lesion can have no effect on the secretion of the medulla If you now stimulate the animal in the region above the section, you no longer can have a reflex secretion of epinephrine If ACTH is secreted under these circumstances then presumably you are dealing with a neural or local neurohumoral mechanism We have done this in various ways One of the simplest ways is to inject a couple of drops of 10 percent sodium chloride under the skin of the animal In the normal animal that results at once in the discharge of epinephrine bringing about a lowering of eosinophiles and of the adrenal ascorbic acid If you inject below the level of section in the spinal rat you get no such response at least so far as this stimulus is concerned There is no immediate release of ACTH if epinephrine secretion is prevented In these spinal animals if you inject epinephrine you promptly get a fall Histamine certainly produces epinephrine secretion, as does cold and many other types of stress That still does not answer the problem of where epinephrine is acting to produce ACTH release As I said before there is also the possibility that it is the metabolic effect of epinephrine that activates the mechanism that Dr Sayers postulates It is possible, as Dr Gellhorn mentioned, although contradicted from his own work that epinephrine might directly stimulate hypothalamic centers or to my mind the possibility still remains it might still act directly

on the cells of the pituitary In the case of muscle epinephrine will cause glycogenolysis in muscle cells which so far as I know do not have the type of innervation one would expect to be a response to adrenergic stimulation That is as far as we are con cerned where the problem stays at the moment Everything seems to point to the importance of epinephrine in the rapid release of ACTH Any neural mechanism that may exist is not nearly as effective in releasing ACTH when epinephrine release is prevented

Thorn May I go on from there with Hume s work and use the same diagram? I think there is one point that may be helpful I assume that everybody has had the experience that ACTH leads to only a transitory lymphopenia Apparently the continued injection must help in the lymphocyte production since one actually sees lym phocytes come back in a good many patients to normal levels verv soon after ACTH has been started The eosinophiles go and stay down and are therefore a more reliable indicator David Hume in the Surgical Department at Harvard has done some experiments in the dog and what he did would support exactly what Dr Sayers did with his transplant He interposed a polyethylene film between the cut hypothalamus and the detached pituitary left in the sella He showed that afterwards there is no vascular or nervous regeneration through the polyethylene but when he injected epinephrine he still obtained a response This indicates that it must either be humoral from this point or else as Dr Long suggests epinephrine may actu ally act directly on the anterior pituitary However if you place small lesions in the region of the paraventricular nuclei and then give epinephrine, you block the effect of epinephrine in terms of produc ing eosinopenia in an otherwise normal dog who continues to respond to ACTH

Long May I interrupt to say that certainly is not so in the rat We have placed lesions in the paraventricular nuclei and the reflex secretion of epinephrine to such stimuli as cold is prevented If you place such animals into the cold they behave much like the demedullated animals In our rats paraventricular lesions or any lesions around that vicinity do not block the action of epinephrine

Fremont Smith What animals?

Thorn Dogs I think you ought to have Hume do this He is a very careful observer and an expert technician These are his results in the dog only with small lesions carefully placed in the paraven tricular nuclei He has gone one step further and I think he agrees that this work is still preliminary If you take an extract of this

area you get a substance which will give all the effects of stimulating the anterior pituitary Other areas of the brain extracted in similar ways and other organs extracted in similar ways do not give that He tells me that actually when you study the cells in the paraventricular nuclei, where he places his lesion they are quite different from normal nervous system cells They actually have numbers of granules and appear secretory That has been reported and is nothing new

Going back to Dr Long s observation, we have had this interesting experience as most of you know, during a major operation you get a fall in eosinophils to zero in four hours If the operation is done under spinal anesthesia you don't get the eosinophil fall until after the effect of the anesthetic wears off That would suggest to me that any central type stimulation from the operation itself is not nearly as important in this mechanism as that via the medullary nervous system pathway from the operative site My own concept today is Hume's, that epinephrine stimulates the anterior hypothalamus and as a result we get a humorally transmitted substance which then acts on the anterior pituitary to induce ACTH secretion This transmitter may be produced also by stimuli from higher centers apparently, so that epinephrine is not the only ACTH stimulator I don't think any of the reported experiments eliminates this concept The capacity to place the lesion exactly right and differences in dog and rat may explain the whole difference between Dr Long's and Dr Hume s findings

Gellhorn How do you explain the experiments with anesthesia which I reported? I think they contradict completely your statement because the anesthesia can very well interfere with the excitability of the hypothalamus What other reason could there be that pentothal will abolish the effect of cold on lymphopenia? We all agree that the lymphopenia is the result of sympatheticocortical discharge

Thorn What is wrong with that?

Gellhorn If I understand you correctly—you said Dr Thorn that adrenalin acts on the hypothalamic region?

Dr Thorn Yes

Gellhorn I believe that

Thorn Pentothal can do one of two things either cut down on the amount of epinephrine released by the medulla or have a local effect in the hypothalamus inhibiting its action to epinephrine

Gellhorn It is possible for instance in the cold experiment

that pentothal would eliminate the excitability of the hypothalamus under the conditions of cold so that the adrenalin is not released You agree with that?

Thorn Yes that would be an explanation

Long There is conflict between Hume s experiment and the one Dr Gellhorn reports Hume states that you can obliterate an area in the hypothalamus which renders the epinephrine ineffective on this mechanism and that the epinephrine effect can only be restored by the injection of an extract from that area

Pincus Is that extract made in such a way to be likely to contain a substance like epinephrine?

Long It might contain a little posterior lobe If it contained any posterior lobe like material we would have of course all kinds of effect from that

Thorn It is taken from tissue far up

Pincus It is an aqueous extract?

Thorn I cannot give you the details of that

White I feel justified in quoting data from a personal letter since the individual who wrote the letter said that he had sent the information off to press I had a letter recently from G W Harris in England and he states that rabbits which previously will respond with a lymphopenia when exposed to an emotional stress do not respond to this lymphopenia when Dr Harris has eliminated or obliterated an area in the hypothalamus

Long That is our experience with eosinophils and Dr Gellhorn s with his barbiturates

White Is the difference Dr Long between the rat and the dog with respect to where the lesion is placed?

Long Maybe That is not the point The difference of opinion between Hume and ourselves is whether when you remove this area in the hypothalamus you not only remove the reflex secretion from the animal such as by exposure to cold but you also prevent the response to injected epinephrine

Thorn If Harris injects epinephrine and gets no effect then?

Long He favors Hume If he injects epinephrine after the lesions he describes and gets a lymphopenia then it is in line with Dr Gellhorn s experience and with our experience

Selye What was the stimulus?

White Emotional excitement

Gellhorn In hypophysectomized animals injected with adrenalin, as far as adrenalin is concerned it is still effective

Sayers Incidentally, I would like to emphasize what has been well established, that epinephrine is discharged in many acute stresses, practically all of them as a matter of fact, and must play an important role in the discharge of adrenocorticotrophic hormone in all of these stresses What we need is elucidation with regard to the exact mechanism of action of epinephrine

Dr Gellhorn spoke of experiments in which he put animals, anesthetized with pentothal, in the cold We presented some experiments a couple of years ago at the Laurentian Hormone Conference, in which we demonstrated that animals exposed to cold exhibit a marked decrease in adrenal ascorbic acid If the animals are anesthetized with sodium pentobarbital and then put in the cold no change in adrenal ascorbic acid occurs An important aspect of this experiment and something which we have thought a great deal about is the fact that anesthetized animals do not shiver and their rectal temperatures go down

Fremont Smith They have no vasoconstriction, I believe

Sayers I presume so

Fremont Smith That is why the temperature goes down

Gellhorn It makes the cold stimulus even more effective

Sayers The unanesthetized animals go through great muscular activity We had postulated that sodium pentobarbital acts through the hypothalamus to prevent shivering the abolition of muscular activity in turn abolished the increased utilization of cortical hormone which normally occurs Dr Ingle has presented evidence which indicates that increased muscular activity is associated with increased need for cortical hormone

Long Let me recommend the spinal animal to you on that point, because with such a preparation you can exercise the lower part of the body without exciting the nerve centers above the section

Fremont Smith It is my understanding that the basic mechanism of the alarm reaction as far as the sympathetic response is concerned is lost with almost all forms of anesthesia One cannot get a fever by means of pyrogens in an anesthetized animal because it doesn t respond with the peripheral vasoconstriction, shiver, etc Likewise these peripheral mechanisms of such an animal will not react to the cold, nor, I believe to anoxia for in this sense the animal is not reacting to the stress situation when it is anesthetized Maybe Dr Selye has something to say to correct me on this

Selye I believe that anesthesia prevents the entire alarm reaction mechanism if it diminishes the stress Whenever it diminishes the stress of course it also diminishes ACTH discharge and all other manifestations of this I wonder to what extent for instance, an animal that has been anesthetized while in the cold would be actually less exposed to the stressor effect of it Similarly an individual in whom the spinal cord is sectioned and then is exposed to trauma in the caudal part of the body, would suffer less stress

Fremont Smith You must define stress Stress with respect to what? I don t think you can say stress as if it meant anything until we define it because the moment you change the animal by cutting the cord or by administering anesthesia you have introduced new stress at the same time as you eliminate or diminish stress of a different type

Selye Nervous stress pain will be diminished Very profound anesthesia in itself will produce corticotrophic stimulation if it is administered long enough

Fremont Smith The point I am trying to make is this that most stress reactions in the normal animal like most reactions consist of reverberating circular processes between environment and the organism *It is not a single event that happens a stimulus followed by a* response but rather an environmental peripheral central, hormonal interaction which is continuous throughout the stress If you anesthetize the animal you have cut out a certain part of that mechanism I feel it important to specify as far as possible what stress or what aspect of the stress one is referring to

Pincus I might at this point give some data which pertains to this which Dr Elmadjian in our laboratory has obtained He has taken rats and exposed them to cold daily over a period of many weeks and from time to time studied the reactivity of the adrenal to administered ACTH, and the results are so incongruous that I am now very much puzzled about this alleged mechanism What he measures after administration of ACTH is adrenal ascorbic acid and cholesterol If you take a rat and give it ACTH the drop in adrenal ascorbic acid is very prompt and the drop in cholesterol is less prompt This was shown long ago particularly by Dr Long and his coworkers If you take an animal that has been in an icebox every day for a matter of three weeks and at this point give ACTH, the result is profoundly surprising The ascorbic acid goes down very slightly and the adrenal cholesterol drops to practically zero

Long Would you mind repeating that again?

Pincus If after an animal has been exposed to cold daily for

about three weeks and you then give ACTH in a dose which causes the typical effect in the normal animal, the adrenal cholesterol drops to almost zero We get values of 0 1, 0 2 percent compared to preinjection levels of around 4 percent The ascorbic acid hardly changes at all

Long That does not surprise me in the least When we worked together at Yale Dr Sayers will remember we pointed out in our papers that there is this dissociation between ascorbic acid and cholesterol There are a number of points about the behavior of the adrenal ascorbic acid that we do not have time to go into this afternoon but I think Dr Sayers will agree with me that one has to use with caution adrenal ascorbic acid as a measure of cortical secretion In many circumstances it is better to use the adrenal cholesterol levels which seem to be much closer to the secretion of cortical hormone If you are using rats I want to point out that you should realize that the rat is an animal which can resynthesize ascorbic acid at a very high rate and in consequence it is not easy to depress the level much below 200 mg percent

Another thing about ascorbic acid with which probably Dr Sayers has also had some experience is that if you put an animal in the cold thus lowering the adrenal ascorbic acid to about 250 mg percent and at that point apply another stress hoping to lower the ascorbic acid even further very little change will occur This is because somewhere around 200 250 mg percent of ascorbic acid is the point at which the rate of resynthesis about balances the rate of discharge Thus if you use the adrenal ascorbic acid as an indicator of cortical secretion in the animal where it is already low you are likely to be misled as to what is happening in the adrenal You may conclude no extra secretion has occured yet measurement of the eosinophils will indicate that it has

Sayers I agree with Dr Long in that regard

Pincus As far as a floor for ascorbic acid is concerned, we all agree The behavior of cholesterol in an animal not under stress is very different from that of an animal under stress The only point that I want to make is that in relation to this general topic of regulation of secretion there is the possibility of a dissociation of these processes extending back to some extent further than we thought

Long If you deprive guinea pigs of all ascorbic acid for 14 days they are still pretty healthy If now given ACTH the adrenal cholesterol falls but not the ascorbic acid since there is hardly any left in

the adrenal The fall in lymphocytes that still occurs indicates that the gland can still secrete its hormones

Pincus May I inquire what you consider the significance of the adrenal ascorbic acid?

Sayers You have opened up the question of the merits of indices of adrenal cortical secretion We must be very careful indeed in interpreting the various indices I would say that adrenal ascorbic acid is of value in acute experiments employing animals in good health in chronic experiments over long periods of time or when animals are subjected to malnutrition then adrenal ascorbic acid changes have to be very carefully interpreted They are influenced by synthesis of the vitamin in the adrenal cortex which in many instances must mask the depleting effect of ACTH As an example, we were studying the effect of malnutrition on the secretory activity of the adrenal cortex and found that the malnourished rats when subjected to stress had much lower adrenal ascorbic acid than the animals that were on an adequate diet At first sight it might be concluded that the malnourished animals had adrenals which were more active actually secreting more cortical steroid but I think the true interpretation is that the adrenal cortical tissue of a malnourished animal is less able to synthesize a corbic acid The concentration of ascorbic acid at any moment depends upon the amount taken up from the blood as well as discharge from and synthesis in the gland itself

Long I believe we would agree that the eosinophil count is perhaps the most sensitive index of cortical hormone secretion

Pincus Dr Thorn made a comment about that I think it is extremely interesting and again I hate to present contradictory data Maybe that is what this meeting is for In schizophrenic patients given ACTH the immediate effect is a very marked drop in the eosinophils If you keep on giving ACTH 100 mg or 200 the eosinopenia disappears and you get eosinophilia a rise up to 500 600 from zero count at injection What does that mean? Maybe it is a matter of 'refractoriness of the adrenals of such persons?

Thorn What is the ketosteroid when you get up to five hundred eosinophils?

Pincus I don t recall the data but the eosinophilia I do remember I can tell you about the urinary uric acid

Thorn What happened to that?

Pincus That is relatively not increased

Thorn That is the whole point I don t think you can use that evidence one way or another Obviously we have seen plenty of escapes with eosinophils I was trying to bring out the point that if

anybody was doing chronic experiments with lymphocytes, particularly in man, that is a disappointing mechanism It has been shown in leukemia and lymphosarcoma that you get involution of tissue but that the rate of production of lymphocytes is greatly accelerated True after the first dip they come back up When you get eosinophilia then I think you are forced to decide whether the gland is activated or not by ACTH It would be different if you were using 200 mg of E and your eosinophils escaped That would be clearer

Long We seem to have accumulated a few ideas for future meetings and one subject would be a more detailed discussion of the effects of adrenal cortical hormones on the white blood cells

CLINICAL STUDIES WITH CORTISONE AND ACTH

ROBERT F LOEB
Department of Medicine College of Physicians and Surgeons Columbia University

I MIGHT CONFESS at the outset that I myself have not treated a patient with ACTH or cortisone that I merely have seen a good deal of work done by others My presentation must perforce be based on second hand information

I think it is fair to say that prior to the spring of 1949 the products of the adrenal cortex and the products of the pituitary which stimulate the adrenal cortex were thought of in terms of substances which might find clinical application in the treatment of adrenal insufficiency With the era of Hench and Kendall our thoughts suddenly began to expand in fields which have not wholly been anticipated On the basis of the observations reported by Hench the first question which came to mind, at least to some of us, was whether or not the rheumatic diseases or rheumatoid arthritis were manifestations of hypoadrenalism which had escaped our detection or whether the effectiveness of the agents cortisone and ACTH, brings about therapeutic result by inducing some measure of hyperadrenalism I don t think that we have as yet a definitive answer on these points and I hope these questions will be discussed later Although we still lack proof I think it is fair to say that most of us have come to believe that in rheumatoid arthritis and in rheumatic fever we are not in reality dealing with significant adrenal insufficiency Certainly patients with overt hypoadrenalism as characterized by Addison s disease are not commonly affected by either rheumatoid arthritis or rheumatic fever Is that a fair statement Dr Thorn?

Thorn We have had a year s background with that aspect of the problem specifically in mind and could not see it

Loeb It is my reaction therefore that some of the diseases in which therapeutic effects are achieved with ACTH or cortisone do not result from hypoadrenalism but that the observed effects are associated with the induction of some measure of hyperadrenalism In other words effects of ACTH or cortisone in these disorders may be ascribed to a pharmacodynamic action of these substances rather than to their normal hormonal effects

To me the interesting features perhaps to be discussed are 1 What

diseases other than those due to overt hypoadrenalism are influenced by these substances? 2 How much do we know concerning the mechanism by which therapeutic results are achieved? With this in mind I have set down a partial list of the diseases *in which* either ACTH or cortisone has been administered It is interesting to me that in *the vast majority of these diseases a favorable therapeutic* effect has been achieved

DISEASES TREATED WITH CORTISONE OR ACTH

1) *Collagen Diseases*
   Rheumatic fever
   Rheumatoid arthritis
   Disseminated lupus
   Periarteritis
   Dermatomyositis
   Scleroderma

2) *Overt Allergies*
   Atopic dermatitis
   Drug sensitization
   Hay fever
   Asthma
   Nasal polyps

3) *Glomerulonephritis*
   Nephrotic syndrome

4) *Gout*

5) *Neoplastic Diseases*
   Carcinoma
   Hodgkins
   Lymphatic leukemia) Chronic
   Myeloid leukemia)
   Acute leukemia

6) *Specific Infectious Diseases*
   Pneumonia—pneumococcal and virus
   Tuberculosis
   Poliomyelitis
   Infectious hepatitis—chronic
   Herpes

7) Cirrhosis of the liver

8) Ulcerative colitis

9) Pemphigus

10) *Hyperthyroidism*

11) *CNS & Muscle Diseases*
   Myasthenia gravis
   Amyotrophic lateral sclerosis
   Muscle dystrophies
   Multiple sclerosis
   Psychoses of various types

Perhaps we can now start with consideration of the beginning of this list *Certainly the first and perhaps some of the most dramatic* effects are in the so-called collagen or mesenchymal diseases including rheumatoid arthritis rheumatic fever lupus erythematosis periarteritis nodosa, dermatomyositis scleroderma and I am not sure that one should not include glomerular nephritis in those diseases

As I have said, I have had no personal experience but a number of people have been working on the possible mechanism of action

Dr Charles Ragan in our clinic made an observation which perhaps may throw light on the problem All of these collagen diseases I think we agree, are associated with overactivity or over reactivity of the mesenchymal tissue in response to some form of injury In the course of treating lupus with ACTH at a time when striae were appearing and the lupus was strikingly relieved three patients developed abscesses others had had incisions of one kind or another for biopsies or what not and Dr Ragan noticed a striking failure to heal and indeed surgeons who looked at these wounds said they could see no granulation tissue appearing In one of the patients, four days after discontinuation of the ACTH, the lupus became worse and granulations appeared in the wound As George Thorn said during dinner and it has been our impression too there is a striking analogy between the effect of ACTH and what transpires in the Cushing syndrome, in which most of us have noted a failure to granulate wounds properly Dr Ragan has recently studied in rabbits the effect of cortisone on the granulation of a denuded ear surface Animals were treated with cortisone for three days before operation and eight days thereafter Sections at five and eight days showed granulation tissue and minimal new blood vessel formation In this situation in the damaged rabbit ear the normal reactivity of the tissues which are also reactive in collagen diseases are almost completely inhibited and the question comes up whether this analogy can be carried over to human disease It seems possible that when one treats collagen diseases the disease is not cured' by eradicating whatever incites reaction in mesenchymal tissues but the *disease picture* which is primarily dependent upon mesenchymal reactivity is inhibited by depressing the reactivity of fibroblasts and perhaps reticulum cells This would account for the reappearance of the disease picture upon withdrawal of the treatment That is as far as this study has gone at the present time

I may say that some years ago because of the failure of wound healing in Cushing s and because of the fragility of the blood vessels we had blood vitamin C determinations done on three patients with that disorder The levels were not zero but they were between 0 3 and 0 5 mg per 100 cc In Dr Ragan s rabbits receiving cortisone there is also a depression of the blood vitamin C level

Perhaps I can go a step farther—and this is all highly speculative I am not at all sure that my anatomist friends can tell the difference between reticulo endothelial cells and fibroblasts as far as the origin is concerned and one wonders perhaps whether these same cells in one form or another may be responsible at least in part, for antibody

production Dr Fischel, at Presbyterian Hospital, studied in rabbits and guinea pigs treated with ACTH, the quantitative passive transfer *of anaphylaxis and the Arthus phenomenon* He was unable to prevent either the passive anaphylaxis or passive Arthus phenomenon employing the techniques of Kabat in those animals In other words, he did not have evidence of inhibition of antigen antibody reaction in these species Furthermore, Dr Knowlton and my wife have been unable in rats to prevent the establishment of nephrotoxic serum nephritis with cortisone At present, Dr Fischel and Dr Bjorneboe of Copenhagen, are studying the possible inhibition of a product of the antigen antibody reaction i e, gamma globulin production following pneumococcus vaccination of rabbits treated with ACTH The indications are that ACTH inhibits the normal increase in gamma globulin elaboration whereas it did not prevent antigen antibody union in passive transfer experiments In the response of collagen diseases to treatment we have, at this time, no knowledge of the fundamental chemical changes, enzymatic or otherwise, responsible for the inhibition of connective tissue reactivity

Another group of diseases perhaps in some way related to collagen diseases and which has shown very definite response to ACTH or cortisone, is the group of diseases in which sensitization is certainly an accepted basis This includes asthma, hay fever and drug sensitization Experience has been quite uniform in that status asthmaticus is relieved by ACTH just as dramatically and temporarily as perhaps rheumatoid arthritis Is that correct Dr Thorn, as far as you know?

Thorn Not in our patients but certainly generally

Loeb In general that has been true

Fremont Smith You had failure?

Thorn Yes I will talk about that later It is a special case

Loeb Also it is of interest that patients with periarteritis with an asthmatic component, have been dramatically relieved In hay fever, the same thing has been reported and the best study on this at least the most concerted study has come from Baltimore though I think Randolph at Northwestern has had analagous results Not only hay fever and asthma but certain drug sensitizations, as already stated, have been strikingly relieved and one of the very dramatic effects with this treatment is the disappearance of nasal polyps I believe polyps are made out of connective tissue too and perhaps their regression under treatment with ACTH becomes understandable In a period of time after cessation of treatment if the underlying basis for the disease persists these polyps recur

Let us consider diseases which improve under treatment with ACTH or cortisone and in which the mechanism of favorable response is even more obscure

In ulcerative colitis I think there are now a number of reports indicating improvement though I think the disea e is one in which caution is necessary as related to interpretation because of its natural history I think one can glean from the reports, that the constituti tional reactions are decreased as they are with other situations where overactive fibroblastic tissue responds to hormone therapy Perhaps again the response may result from the suppression of mesenchymal reactivity

Max Finland reported in Chicago* on two patients with pneu mococcus pneumonia treated with ACTH The administration of ACTH terminated the febrile response and was responsible for clearing of the lung but in both of these patients whose temperature came to normal promptly, positive blood cultures persisted for approximately 48 hours in the absence of fever This raises a very entertaining point as to whether the mechanism for the disposition of the penumococcus i e, phagocytosis has been somehow interfered with in this process of preventing reactivity of tissues In primary atypical pneumonia the constitutional manifes tations of the disease were relieved on two or three occasions with relapse of febrile reaction upon discontinuation of ACTH

I am really not in a position to comment on patients with pemphi gus except to say that rumor has it that they are strikingly responsive to ACTH

Now we come to a disease which is quite apart from these inflam matory disorders and that is hypertensive vascular disease A num ber of investigators have commented upon the fact that a fair per centage of people receiving ACTH, and particularly those with some hypertension have a rather striking and at times terrifying increase in hypertension which may culminate in cardiac failure with pul monary edema or in cerebral accident One patient developed rather severe hypertensive encephalopathy at the New York Hospital We had one with a rapid rise in blood pressure further complicated by subarachnoid hemorrhage With the administration of cortisone in doses of 80 mg a day to hypertensive patients just is with the administration of the cortical extract Dr Perera found there was a small but definite tendency for the blood pressure to fall in the course of time He has one patient at the present time who has been on cortisone dosage level of 200 mg a day for now approximately three weeks This patient had a very transient rise in blood pressure

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* Proceedings of the First Clinical ACTH Conference edited by John R Mote Blakiston Co Philadelphia 1950

for two or three days, following which the blood pressure has continued to fall and has come to normal levels I think there are a number of possible explanations which do not justify speculation at this time

Next we come to the problem of neoplastic diseases and many of us have heard Dr Pearson from Memorial Hospital talk at one time or another You have probably seen his dramatic pictures showing recession of lymphoid masses in lymphatic leukemia and in lymphosarcoma As Dr Thorn indicated this afternoon, in the leukemic patients this shrinkage of lymph nodes was associated with an increase in the circulating lymphocytes in the blood Whether this represents a stimulation of lymphocyte formation or whether it may represent a release of lymphocytes through dissolution of supporting tissue we do not know at this time Be that as it may, I believe Pearson would be the first to say that he has no evidence of cure The lymph node masses and spleens have remained smaller anywhere from two or three weeks to as long as two or three months That is interesting

Fremont Smith Two or three months after cessation or during continuous treatment?

Loeb After cessation of treatment I cannot tell you whether the long sustained remission was after cortisone or ACTH, or whether it made any difference which one was given In carcinoma of the prostate I think the Memorial Hospital results have been entirely negative

As far as childhood leukemia is concerned some of you who were at the Chicago conference can perhaps remember better than I what Taylor reported I believe he said remission had been induced with ACTH in one acute childhood leukemia

The reaction of myasthenia gravis is peculiar The group at the New York Hospital have reported that in their series of some eight patients treated with ACTH for five days patients seemed to get worse They then stopped the hormone and thought they induced spontaneous remission from there on On the other hand, I believe none of those patients was free from the administration of prostigmine even in their so called period of remission I think it would be worthwhile hearing from Dr Conn Dr Thorn and others of their experience with this treatment in myasthenia gravis and whether the worsening during the period of hormone administration was associated with the potassium loss I don t think the latter has been definitely established, but I think it is a real possibility

As regards congenital hypoglycemia McQuarrie has reported that ACTH alleviated the disorder in a child or children I believe they

stayed free of the hypoglycemia for months after ACTH was discontinued The condition is a puzzling one

Thorn He had to continue ACTH but it was in very, very small doses

Loeb He did keep on? Then there is less of a puzzle

Long He let it go for a while and started with small doses, something like 2 5 mg

Thorn Five mg per day

Loeb I am relieved Then there was not a miracle in the fact that they stayed cured

As far as the problems of hypothyroidism and gout are concerned we have had very limited experiences with both

The problems of the effects which one sees in the diseases mentioned are vastly complicated by virtue of the fact that ACTH and cortisone have so wide a spectrum of physiological activities including those on electrolyte behavior, glucose metabolism effects on nitrogen excretion enzyme activities and others too well known and too numerous to review The correlation of the activities with therapeutic effects constitutes a great challenge

DISCUSSION

Long Well, gentlemen there is an amazing story at least in its beginnings as Dr Loeb has said It is extremely interesting We have heard a great many things about the physiological effects or known effects of adrenal cortical hormones and in this list of diseases except for chronic hypoglycemia nobody feels that they can put their fingers on the reasonable explanation of these effects I am certain Dr Loeb and many other people will have to handle these situations in the patient I am grateful for any suggestions you can give as to actually what happens when you give a patient with rheumatoid arthritis cortisone or ACTH

Conn I would like to bring up one question for discussion It is my feeling that so far as the rapid clinical responses to either cortisone or ACTH are concerned that those clinical responses may occur many hours and sometimes two days before any measured metabolic effect is discernible This makes me feel that chemically and physiologically we are not measuring the right things yet It is probable that early changes in enzymatic activities are occurring before we observe gross metabolic changes

Long The eosinophil change does that not occur promptly?

Conn No not even that I was talking to Dr Thorn about that

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reaction then that enzymatic reaction to begin with is going in an abnormal way

Loeb That would be my guess

Long Somebody mentioned the relation of the cortical hormones to hyaluronidase This is one of the newer pieces of work on the effects of cortical hormone, which at the moment does not appear to fit anywhere in the things we are discussing today There is apparently a marked inhibition of hyaluronidase by these hormones The relation of hyaluronidase and hyaluronic acid to the so called collagen diseases has been discussed for some time Now we find that the substances that are most effective in relieving rheumatoid arthritis are also the most effective in inhibiting the action of hyaluronidase What the relation of hyaluronidase and hyaluronic acid is to these disorders of the mesenchymal tissues we do not know It is evidently of some importance Dr White would you care to comment on this?

Loeb May I comment on these and more recent studies? Dr Opsahl (Dr White correct me if I am wrong) injected a small amount of India ink into the skin of normal mice and found that the ink was sharply localized

When you and she injected hyaluronidase with the India ink there was an area of spread approximately twice the area of the normal injection In the adrenalectomized mouse there occurred a considerably larger area of spread of India ink by itself If you added hyaluronidase to that, you got an enormous area of spread in the adrenalectomized mouse The addition of or the pre treatment of these mice with cortical extract in this experiment caused a striking decrease in the area of spread of the India ink Dr White and Dr Opsahl also found that DOCA had no effect on the spread in contrast to the effect of cortical extract An Englishman Dr Spencer working in our Department with a little more quantitative modification of the method confirmed Dr White s and Dr Opsahl s finding about the effect of hyaluronidase in the normal and adrenalectomized mouse He also confirmed the fact that desoxycorticosterone had no inhibitory effect on this spread whereas pure cortisone had an incredibly great inhibiting effect on the spread That was treating the mouse with 1 mg of cortisone 24 hours before and 1 mg six hours before injection Compound A given twice in doses of 3 mg in other words in three times the dose of cortisone had virtually no effect on the spread of India ink with hyaluronidase In other words there was rather good correlation with the inhibition of spread by hyaluronidase between the one substance which is active in rheumatoid arthritis and the other two steroids which are inactive in rheumatoid arthritis

a little while ago With cortisone as we get it now (200 mg of cortisone a day), the response in arthritis occurs within hours but the eosinophil change has not occurred for as long as 48 to 72 hours after the administration of the first dose of cortisone Incidentally, cortisone in that dosage, 200 mg a day, fails to drop the eosins anywhere near the level resulting from 50 mg of ACTH in the same individual With ACTH you get a very prompt response but not with cortisone We wondered whether the insolubility of the material was a factor in the slow response of some of the things that we were measuring The reason we thought it might be a question of solubility and increasing concentration was the continued effect of cortisone for three or four days after the material was stopped so far as the metabolic aspects were concerned Our feeling has been that the rapid clinical improvement in arthritis for example, is probably not due to quick dissolution of fibrous tissue but to a very rapid change in some enzymatic activities which we were not measuring, and that the metabolic effects of those enzymatic changes were not evident for several days

Pincus Is this true of every index that is commonly taken for example urinary uric acid?

Conn Yes The things we have been following have been electrolyte metabolism, blood and urinary uric acid blood glutathione blood and urinary sugar, eosinophils sweat electrolytes, nitrogen metabolism urinary steroids serum cholesterol and others Sweat, sodium and chloride incidentally falls as the result of cortisone activity It does not begin to come down until about the sixth day with cortisone It comes down on the second day with desoxycorticosterone or ACTH

Loeb Dr Conn, I don't think that fibroblasts themselves are 'poisonous' but the factors that cause them to react and the products of reactivity may perhaps be poisonous' After all, if you get proliferation of tissue you are probably dealing with enzyme systems which make tissue grow I assume we must invoke protein synthesis to make new cells This may be a reflection of the capacity of these tissues to respond by virtue of some biochemical stimulus the nature of which we are wholly unaware The growth of mesenchymal tissue or its inhibition is measurable and the inhibition appears to correlate with improvement in the disorders characterized as 'collagen diseases' I am not sure that that is very clear but the idea is that the hormones under discussion appear to block something which gives mesenchymal tissues the capacity to react and it is the products of their reaction which make the disease picture

Conn Yes and if that thing that is blocked is an enzymatic

of the C-11 compound and the 11-desoxy compound was very interesting The 11-desoxy compound increased the rate of water passage through the wall under the influence of hyaluronidase Dr Opsahl has gone on with this work and she has some experiments which would bear out at least in preliminary experiments, this *in vitro* effect on hyaluronidase She has measured the rate of appearance in an *in vitro* system of reducing sub tances resulting from the action of hyaluronidase on hyaluronic acid If you then add various amounts of cortical extract you can get as much as 40 50, 60 percent less reducing substances which under standard conditions also indicates inhibition of the enzyme system by the hormone This is in agreement with the experiments of Seifter and his colleagues that an effect can be demonstrated *in vitro* as well as *in vivo*

Ingle Have steroids, which do not have cortical hormone activity been tested as control substances?

Long Seifter tested quite a number of steroids and Dr Opsahl has also tested a number of them in her system None had any effect except cortisone Cortisone is the one substance that stands out far above all others in its ability to produce this effect

Loeb Cortisone blocks this Compound A does not block it DCA does not block it

Bloch What is the source of hyaluronidase?

Long Testicular

Bloch It is interesting to see the effect from other sources

Long The streptococcal hyaluronidase for example

Thorn One phase of treatment with ACTH that is never brought up, is that in all of these patients you are injecting a pituitary protein which based on our experience with all other pituitary extracts is a distinctly antigenic substance Yet nobody seems to be at all excited that these patients respond over and over again, and that the number of local reactions is extremely small As we inject thi material there should be an antigenic response to this preparation, but because of the fact that this particular substance causes the secretion of a tremendous amount of adrenal cortical steroid during the period of injection allergic reactions are prevented We have had this experience After you stop injections two or three days later we have observed patients develop all sorts of weals and reactions when the level of cortical steroids was down

For example suppose an individual is started for the first time on ACTH and we measure the response to a dose of 40 or 100 mg The individual is treated for a period of time and treatment is then interrupted for about 2 weeks Then a second period of treatment i initiated and the response as measured by the same tests as used

Long May I point out that this is a physiological effect that the amount of spread you get is determined by what you do to the animal If you put the animal in a temperature of 85 or 86 degrees you get an activation of the adrenal cortex and much less spread of the India ink injection than you do if the animal is kept at 70 degrees, so that these factors produced augmentation of adrenal secretion There appears to be a correlation between the conditions that provoke cortical secretion with those that restrict the spreading phenomena

Pincus This phenomenon was described by Menken a number of years ago and he found that the inflammatory reaction was inhibited by adrenal extract

Loeb Yes Menken, I believe, used adrenal extract Isn't that right?

White Yes He used adrenal cortical extract

Long He used his necrotizing factor at that time too

Pincus Leukotaxin

Loeb He did not do the India ink spreading study

Pincus He did not try the effects of hyaluronidase

Long Some of you may have seen the paper by Seifter (Seifter, J, Baeder, D H and Dervenis A Alteration in permeability of some membranes by hyaluronidase and inhibition of this effect to steroids *Proc Soc Exper Biol & Med 72 136 (1949)*) which reached my desk last week He takes the bladder of the rabbit turns it inside out ties it on the tube puts a 10 percent sucrose solution inside the bladder and immerses it into distilled water He has thus prepared an osmometer by which he measures the rate at which water enters the interior and rises in the tube If hyaluronidase is placed in the solution outside the bladder—remember the mucous membrane is facing the hyaluronidase—the rate of rise in the osmometer is increased Now if you add adrenal cortical extract or compound E to the external fluid along with hyaluronidase the rate of flow is decreased On the other hand if desoxycorticosterone is placed outside along with the hyaluronidase the rate at which the water passes through the bladder wall increases even when the bladders are dried at 100 So there was no question of their loss of viability any more the same effects are observed with the dead membrane This is an important point because it indicates that this effect on hyaluronidase is probably directly on the enzyme and is not related to living cells In other words the action of the hyaluronidase on the substrate whatever it is in the bladder wall is inhibited by the presence of cortical hormone It did not matter whether the bladder was alive or dead but the antagonism or the opposite effect

negative nitrogen balance That is conditioned as Brown and others have shown so nicely, by the previous nutrition of the patient

Lastly, in most patients with lupus that have been treated, and certainly in our experience to date in glomerular nephritis we are totally unable to affect the proteinuria I have often wondered why it is that, with all the beneficial results, proteinuria does not disappear with this form of treatment We have seen red cells disappear from the urine on two or three occasions but we have rarely seen the protein disappear, although others have reported it

Pincus Have you ever tried the combination of testosterone and cortisone?

Thorn Not in these experiments I would not quite go along with Dr Conn I think there are a few measurable things in which you can detect changes in the first few hours particularly after ACTH What strikes me as a most interesting phenomenon immediately after ACTH is the change in the patient s outlook and the drop in temperature The temperature may be 104° in the evening and it will have fallen to 99° by the next morning This is a predictable result of therapy His joints will still be swollen but the temperature is down and the patient is euphoric Finally, if you treat many of these patients long enough you do see psychoses develop in some of them I think that is a very serious long range complication So the problem of complications comprises that of Cushing s syn drome the problem of potassium depletion (which is severe but which can be corrected) and the problem of inducing a psychosis It is interesting that you have an almost aspirin like effect when you give ACTH, which I think does not seem possible of explanation by any of the mechanisms which we have discussed so far

White May I ask Dr Thorn whether there was any indication that the patients on the low protein intake differed in any way from the patients not on a low protein diet with respect to their sensitivity in response to cortisone? Was there any intensification of the pro teinuria?

Thorn No intensification Our situation was quite different from that reported by Farnsworth We were not dealing with edematous patients trying to set in the cycle of diuresis which we often know is followed by a loss of proteinuria These were patients with the nephrotic syndrome who had been studied for a year They had a background of proteinuria and had been on a low protein diet for a period of time

White I was thinking about the experimental proteinuria reported by Dr Addis As many of you know Dr Addis reported the pro duction of proteinuria in rats given kidney renin This work is

during the first period of therapy, turns out to be somewhat disappointing I think your first thought is are you dealing with anti hormone or antibody or an adrenal that is tired? As the treatment goes along you soon get back to the original response suggesting that if you can hurdle this initial production of hormone you ultimately take care of these blocking factors Leathem, at Rutgers University, has assayed serum for us and we find high levels of antibody in such patients We have had great difficulty getting anyone to support studies in terms of testing the nature of the antibody to ACTH, but I am convinced that antibody formation is involved and the only thing which saves us in this situation is this chain reaction which tends to dispel the inhibition

Another point, to go along with Dr Loeb, is this if these diseases represented any degree of adrenal cortical deficiency, why do we have to produce Cushing's syndrome in order to cure them? For practical purposes you have to produce Cushing's, the only patients in whom you don't produce Cushing s are those that you do not treat long enough In the asthmatic, or in the patient with skin disease which isn't cured in a week, when you have to go to four to six weeks of treatment, you almost invariably get Cushing's with the doses that are used

Another interesting fact is the effect of treatment with cortisone on ACTH response First of all, before you start treatment, you do an ACTH test and you define the response in terms of eosinophils, uric acid and the 17 ketosteroid excretion You treat for two weeks with 100 mg of cortisone You then stop treatment For two to five days you will fail to get an adequate adrenal cortical response to the ACTH test In other words that adrenal has atrophied and it won't be brought back very easily by the ACTH during that period I don't know just how long the period lasts We have had cases that we are trying to follow You can see after you stop the cortisone treatment that you are going to have the widest possible swing, because within two or three days you are going to go from essentially a Cushing's down almost into an Addison s range of adrenal secretion You just hope nothing happens to the patient during the week after you stop your cortisone therapy On the other hand if the disease can be modified by shock therapy this is about as good shock therapy in terms of wide swings in adrenal hormone that you can possibly get You do go into a temporary atrophy after ACTH itself, but I don't think the swing is nearly as great as it is with cortisone

Finally, if you place a patient on a low protein diet for some period before you undertake this form of treatment and we have had two such patients, the administration of compound E does not cause any

hypertensive heart disease is practically unknown in the schizophrenic There is no such thing as asthma in schizophrenics Just to give you a dramatic instance of that we had one patient at the Worcester State Hospital with marked schizophrenic symptoms who recovered after electroshock therapy but then developed a terrific asthma When he returned again to the hospital with his psychosis as a lot of these people do the asthma was gone With perhaps the exception of pneumococcal infection which I think is dubious most of the diseases on Dr Loeb s list—certainly the collagen di eases and the allergies—are extremely notable for their absence in schizophrenics

Secondly, in a proportion of the patients with schizophrenia we have observed a reactivity to ACTH very close to the normal In another proportion of these patients there is no indication of a normal reactivity, at least in the 25 mg acute test We are of course dependent as mo t of you know upon diagnoses which are difficult to make and are sometimes questionable Recently we have been giving much larger doses of ACTH to schizophrenics and here we can differentiate on the basis of symptomatic changes in these patients In the five for whom we managed to get enough ACTH, two showed some remission of the psychiatric symptoms and three did not Of the three that did not react to ACTH one showed the sort of psychotic behavior that you are talking about He seemed to develop the equivalent of an agitated depression and this is apparently unusual in a schizophrenic I think that we still cannot talk about psychoses or even psychiatric disease What we have to talk about is something which is unfortunately psychiatrically ill defined and which certainly is not a unitary matter I would like to know the steroid picture in the case of those individuals receiving ACTH who develop the depressions

With cortisone even with ACTH the indication is that we should search for the abnormal production of certain steroidal substances I would like to mention some studies that we have recently completed First the analysis of the ketosteroid fractions of the urine show that certain substances are missing in the schizophrenic s urine which are present in normal urine What this indicates from the point of view of production of steroid, I don t know The fact that certain things are not present makes us suspicious that there may be a difference in the nature of hormone synthesis

The second observation is that of Dr Hoagland (Hoagland, H Callaway E Elmadjian F and Pincus G Adrenal cortical responsivity of psychotic subjects in relation to electroshock treatments *Psychosom Med* (in press) (*1950*)) who has recently completed

being continued largely by Drs Howard Goodman and Jesse Marmorsten at the Cedars of Lebanon Institute for Medical Research, Los Angeles, California The proteinuria produced by renin appears to be *dependent* upon the presence of the adrenal

Thorn One hundred milligrams of cortisone per day did not increase the proteinuria in two clear cut cases with proteinuria Patients on ACTH (40 to 100 mg a day) showed no significant rise in proteinuria

Loeb May I ask a question which is a little off the path here? Dr Thorn has commented on psychoses I wonder if psychoses have been observed with cortisone as well as with ACTH?

Thorn The Mayo Clinic noticed this in one patient with cortisone

Long I believe it was quite severe

Thorn Our most severe case whom we had to commit, was in a patient with lupus The sad thing is the lupus is cured at the present moment I asked Derrick Denny Brown whether one could visualize the cerebral manifestations of lupus being aggravated Obviously the clinical picture in this case was not different from that which we sometimes see in untreated lupus, but the psychotic manifestations came on when the rest of the disease was improving

Pincus Was the effect observable during the course?

Thorn In the course of ACTH administration

Ralli I was wondering if the type of psychosis produced would respond to electroshock therapy

Loeb I can answer that One patient we had developed a severe agitated depression while on ACTH This continued after withdrawal of the steroid The patient was then given electroshock therapy and after four treatments was restored to normal

Thorn Interestingly enough early in the psychosis when we stopped ACTH and when the temperature went up from the disease the psychosis became very much aggravated with the hyperexia When we gave ACTH again the patient became calm after the earlier very restless agitated state

Pincus Perhaps I ought to comment briefly on the question of adrenal function in the psychoses, because there is obvious disagreement on this point The only psychoses we have studied to any extent have been the schizophrenic psychoses The picture is different from that present in the agitated depressions and according to the psychiatrists there is no question as to a distinction between the two types of disease We have inquired recently as to the incidence in schizophrenic individuals of the types of conditions listed here by Dr Loeb and we find that practically every disease which responds to ACTH is absent in the schizophrenic psychoses For example

which she saw occurred when she gave the adrenal cortical extract intradermally locally either at the same site as the enzyme or mixed with the enzyme Under these conditions spreading was totally inhibited the effect was much more striking than when the adrenal cortical extract and the enzyme were injected at different sites I might say for purposes of historical correctness of the record that in 1943, while Dr Dougherty and I were at Yale we were led initially to relate hyaluronidase to the adrenal cortex as a result of reading the review by Dr Duran Reynals, and then discussing the problem with him As you know Dr Duran Reynals discovered the testicular spreading factor, now known as hyaluronidase Dr Dougherty and I were impressed by the fact that in his review in *Bact Rev* 6 *197* (*1942*) on tissue permeability and spreading factors in infection contribution by host and parasite problem Dr F Duran Reynals listed a large number of unrelated stimuli physical and chemical which inhibit the spreading factor Since these same stimuli also augment pituitary adrenal cortical secretion a relationship of the adrenal to spreading factor inhibition suggested itself About this time also the effect of salicylate injection on inhibition of spreading factor was described The fact that the dose of salicylates which was necessary to inhibit hyaluronidase *in vivo* was so much less than the amount required to inhibit *in vitro* suggested to us that salicylate might also be a non specific pituitary adrenal cortical stimulant After discussing the matter with Dr Duran Reynals, he provided one rabbit into which we injected intradermally India ink and testicular extract The next day the rabbit received 10 mg of ACTH subcutaneously and 6 hours later the India ink and enzyme again were given intradermally on the opposite side The limitation of spreading following hormone was striking As a matter of fact Dr Duran Reynals enthusiastically skinned the rabbit and mounted it on a large cardboard base which probably still remains in one of the laboratories at Yale Nothing more was done until 1947 when Dr Opsahl came to the laboratory and the problem was taken up again That summer we ran through about 1000 mice with results which Dr Opsahl has now published and Seifter has recently confirmed I may add that Dr Opsahl s discovery of the inhibitory effect of elevated environmental temperature on the spreading phenomenon occurred accidentally when the thermostatic control failed in the animal house during a three day period that summer During that time the control animals showed no spreading whatsoever even when injected with testicular extract This was interpreted as non specific pituitary adrenal cortical augmentation

a study of schizophrenics receiving shock therapy Before electro shock the patients were given the 25 mg ACTH test To make a long story short, the four patients who showed sufficient remission to be sent home on visit were the ones who showed good response to ACTH, and the five who remained in the hospital were those who showed poor response Possibly we should redefine the psychiatric state of schizophrenia in physiological terms We are now dividing patients with schizophrenia into those who respond to ACTH and those who do not respond

We also have found that the acute cases tend to show greater responsiveness to the ACTH than chronic cases I think that the nature of the adrenal involvement in these diseases requires a lot more investigation The suggestion of a qualitative difference in secretory activity in certain schizophrenics as compared to normal *persons is something which we hope to get at analytically by direct* steroid analyses

Thorn In such studies, you have to be very careful to know that you are dealing with the same batch of ACTH I think the person ality of the patient is the biggest factor as to whether or not he is going to develop a psychosis with ACTH That is an important thing to watch for and there are certain individuals who because of our experience are not given any prolonged treatment with ACTH

Loeb That is the feeling of our psychiatric group They think they may be able to spot those who may get into trouble

Thorn Going back to Dr Conn's question of the time element involved in the measurements which indicate a response to ACTH, I do know that within an hour after a dose of ACTH or cortisone you can show a change in the radioactive iodine uptake on the part of the thyroid That is within an hour I just don't know how soon changes begin to occur

Conn That change in particular is an enzymatic reaction isn't it?

Thorn Yes

Conn It would seem that in the very earliest phases we must study more subtle indices of enzymatic activity if not the enzymes themselves Dr White is hyaluronidase dependent upon sulfhydril groups? Is that one of the so called SH, enzymes?

White I don t know of data on this point The enzyme has not as yet been obtained in pure form I believe a very good biochemical task at the moment is the purification of hyaluronidase one will be in a better position to decide whether the steroids have a direct effect on the enzyme The fact that they have a direct effect was suggested in the early studies of Dr Opsahl certainly the most striking effect

Thorn E in large doses will raise 17 ketosteroid excretion whereas in small amount it will not

White Don t you think that this is a big jump? Do you think then that in the Addisonian who has a diminished adrenal cortical function, and whose adrenal cortex is therefore probably not contributing to a normal production of androgenic material, there is an excessive stimulation of the production of androgenic material by some other tissue?

Loeb While we are speculating let me give another theory I suppose it is proper to quote Dr Sprague s comments made concerning Albert s observations on the action of ACTH Albert had injected ACTH in green frogs and made them turn brown I believe that he felt there was probably some contamination in the ACTH which was stimulating chromatophores One might say that in the Addisonian having no adrenal to respond there is increased pituitary secretion including a substance which stimulates pigment deposition in the skin

Thorn That is right but give compound E and the Addisonian becomes more pigmented That is what threw me off

Pincus In the Addisonian the intermediates may be affected differently

Ralli We studied hypophysectomized black rats and they also pigmented due probably to the atrophy of the adrenal cortex that occurs after hypophysectomy

Loeb That does not help my theory at all

White In the tissues which Dr Dougherty subjected to very careful histological examination in connection with animals given ACTH or the 11 oxygenated adrenal cortical steroids there were produced profound histological effects in terms of the wide varieties of cells and tissues which were affected One wonders whether you have one of these rare relatively widespread acting substances in the sense that its action is not limited to a single type of response With this in view there may not necessarily be a common mechanism underlying the effective therapy in all of these diseases Dr Selye perhaps has had more experience than anyone in terms of the histological effects of steroid hormones I am not speaking about large doses but about relatively small doses of these substances There is a bone marrow effect There is a kidney effect There is an effect on mature lymphoid tissue There is an effect on primitive reticulum There is an effect on fibroblasts and perhaps these are just a diversity of physiological responses rather than some common underlying mechanism This of course does not help explain the mechanism of the therapeutic effect in any one of these cases Would you like to comment Dr Selye? I would be interested to get your reaction

Long I think Dr White is giving you a very good idea of how we make these discoveries at Yale

White I might say, incidentally also for the benefit of the record that hog ACTH was first prepared in pure form at Yale by Dr George Sayers, who was a graduate student in my laboratory at that time and not by me The publication by G Sayers, A White and C N H Long appeared in the *Journal of Biological Chemistry, 149, Aug 1943* in the same issue as a paper by C H Li, H M Evans and M E Simpson, who had independently isolated ACTH *from sheep pituitary glands*

Pincus The ACTH we first used was prepared by Dr White

White I think it would be very worthwhile if perhaps at a future conference Dr Duran Reynals might review the spreading factor field He is perhaps the most capable person in the world to discuss the physiological significance of the intracellular cement substance

Long I think we should have them all down here, Dr Seifter, Dr Opsahl and Dr Duran Reynals *We will hear a lot more* about the hyaluronidase effect if it is correct that the enzyme is inhibited by cortical hormone *in vitro*

Thorn May *I change the subject* for one moment to *another* system? Why does ACTH and why does compound E increase pigmentation in all these patients and also in the Addisonian when you use cortisone? We have implanted E pellets time after time in the Addisonian whose condition was stationary, who had nice white scars, and we got pigmentation It has also been reported with ACTH

Ralli Does ACTH depress the secretion of desoxy like factors? When we take the adrenals out of black rats we can prevent the deposition of melanin in the hair follicles and bulbs by injecting DOCA If the administration of DOCA is begun right away it inhibits the pigmentation Someone else made use of this observation and applied DOCA directly to the skin and inhibited the growth of hair This report was published by W L Whitaker and B L Baker (*Inhibition of hair growth by the precutaneous application of certain adrenal cortical preparations* *Science* 108 *207* (*1948*))

Pincus Dr Thorn, do you recall patients tanning more easily?

Thorn *They tan very easily*

Pincus It seems like an androgen effect

Pincus Compound E should be easily transformed to a C 19 steroid You get an increase in the 17 keto excretion This may be just an androgen effect

duced by an excess or imbalance between these two types of secretion? That is, if for one reason or another the gland is producing more of the 11 desoxy type, then you will expect to find this type of change taking place I have often wondered why, if such an imbalance does exist we do not see other signs of overdosage with the 11-desoxy type compounds Why do you not see the marked effects on electrolytes that are so readily produced by DOCA injection? I believe you have also reported that you can produce these changes by the injection of lipolyzed anterior pituitary extract into sensitized animals We have heard today a great deal of discussion as to the kind of steroids that are liberated from the adrenal by ACTH and the impression I gain from the discussion is that the greater part of the secretion consists of C 11 compounds I think you mentioned in your discussion this afternoon that you had found that cortisone *itself under certain conditions would produce in the sensitized* animal the type of lesion that you describe If I am not correctly presenting your hypothesis perhaps you will tell me

Selye Before answering that I am not sure I understood the first question why do we not see more often desoxy overdosage symptomatology? You mean in patients treated with desoxy?

Long No If a patient develops rheumatoid arthritis and if this arthritis in man is produced in the same way or by the same process as produces similar lesions in the rat given DOCA why did we not see the other effect of overdosage with desoxycorticosterone in these cases?

Thorn There is one point which is helpful in your theory and that is when you deal with Addison s disease you don t have to worry about administered desoxy inhibiting the secretion of the 11 oxysteroids While we don t see rheumatoid arthritis (at least I have not), nor periarteritis nor lupus in all Addison's disease we do see a stiffness develop in our patients treated with desoxycorticosterone and that stiffness in some cases goes so far as to show actual calcific changes in the ears They get hard The fingers look like early scleroderma the Raynaud type of change and the muscles all have a great amount of stiffness which you can change in 48 hours by giving whole extract This stiffness looks like joint disease but we have never been able to show any *joint disease*

Selye I think with this preliminary discussion I can now attempt to answer the question put to me by Dr White

First let me say that we certainly have no definite evidence indicating that two different compounds (mineralo corticoids and gluco corticoid) are actually secreted into the blood as such However

Selye I have not very much to say about this but before I say what little I can, I would like to ask another question of anybody right here who feels like offering an answer Do you feel that there is any relationship between the cure of the collagen diseases by ACTH and cortisone and the production of these same diseases in experimental animals by desoxycorticosterone or is it your impression that this is purely accidental?

Sayers I would say yes, Dr Selye We have evidence that when we administered large doses of DOCA we were producing insufficiency of the cortisone like compounds It was for that reason we started the experiments which I talked about this afternoon in giving DOCA and ACTH simultaneously We certainly have very suggestive evidence that ACTH will counteract the effects of DOCA in producing certain pathological lesions We can definitely say that ACTH does not aggravate the lesions produced by DOCA This is definitely of interest in relation to the findings in the collagen diseases where an excess of ACTH or an excess of cortisone, is having therapeutic effects

I would certainly agree with Dr Loeb and Dr Thorn that what we are dealing with in man is a pharmacological action rather than a physiological action, so perhaps the parallelism is not absolute However, DOCA may very well predispose the animal by inducing a deficiency

White What do you do about a situation in which the same hormone when administered can either ameliorate or exacerbate a condition?

Sayers What do you have in mind?

White Gout

Thorn It does not exacerbate gout

White I am thinking of the report of Hellman

Thorn Hellman s exacerbation came after he stopped therapy He never saw gout made worse when on the hormone

Long If I might say a word to Dr Selye's question, I hope that at a later date we might be able to devote a little more time to his question instead of endeavoring to give a yes or no answer at this time The whole question of antagonism if there is antagonism between desoxy compounds and the C 11 compounds, is a very important one certainly worthy of more discussion than we can give it this evening Just one question Dr Selye and—if I am making a misstatement about your views I know you will correct me at once—is it your feeling at the present time that in the adrenal we have two types of compounds the C 11 and the 11 desoxy compounds and that many of the e collagen types of di eases are pro

the concept of the "diseases of adaptation" Even desoxycorticosterone itself (whose hypertensive nephrosclerotic, periarteritis producing and other "hyalinosis" actions have now so extensively been confirmed in various laboratories) is entirely ineffective in producing any of these changes in animals kept on a sodium free diet Yet, it retains its adrenocortical atrophy producing effect and its unfavorable action upon the development of arthritis irrespective of the salt intake Thus variations in this one conditioning factor (sodium) suffice to cause qualitative alterations in the syndrome of mineralo corticoid intoxication It is not within the scope of this meeting to discuss all the "conditioning factors" which can induce qualitative alterations in the effect of ACTH, cortisone and other corticoids but if we disregarded their effect it would be impossible to understand how exposure to stress could produce such a variety of diseases through the same hypophysis adrenal mechanism

Perhaps one of the most important pertinent facts is that if mineralo-corticoids and gluco corticoids are simultaneously administered to an animal the latter inhibit only some, and increase other manifestations of the former Thus we found that rats receiving toxic doses of desoxycorticosterone do not develop periarteritis nodosa if they are simultaneously treated with cortisone However the latter hormone does not inhibit the renal damage produced by desoxycorticosterone so that the symptoms of intoxication are dissociated Conversely, the intense lymphatic atrophy caused by cortisone is not prevented by desoxycorticosterone and the adrenal atrophy produced by both of these steroids (though much more intensively by cortisone) is even more pronounced if the two steroids are simultaneously given In this respect there is an actual synergism between cortisone and desoxycorticosterone

Dr White commented upon the fact that the corticoids affect so many organs We wonder whether this could be due to an action upon membrane permeability itself The concept of Eppinger—according to whom derangements in membrane permeability play an important role in the development of what we now call the hyalinoses or collagen diseases—has received little attention by those interested in corticoids It will be recalled that according to Eppinger increased membrane permeability leads to what he termed "serous inflammation", with the discharge of potassium from the cells and its substitution by sodium At the same time the intercellular fluid and electrolyte content increase Perhaps some "diseases of adaptation" could result from derangements of the antagonistic action of mineralo corticoids and gluco corticoids upon this increased membrane permeability which appears to result from any kind of stressor

there is excellent evidence to show, that in addition to the gluco corticoids, compounds possessing mineralo corticoid activity are present in the adrenal cortex under normal conditions The amorphous fraction of Kendall, the so called "sodium factor of Frank Hartman, is apparently endowed with typical mineralo corticoid potency and seems to be practically, if not completely, devoid of gluco corticoid actions Up to now the amounts in which these mineralo corticoids were available did not suffice to prove that they actually cause hypertension and hyalin disease (*periarteritis nodosa, nephrosclerosis* etc) in animals However,—as I have stated earlier during this conference—desoxycorticosterone (or Reichstein's compound S), which differs from cortisone only in that it does not possess an oxygen on carbon 11, produced marked hypertension diuresis and hyalinosis in rats sensitized by unilateral nephrectomy and a high sodium diet There appears to be little doubt about the fact that desoxycorticosterone is a normally occurring adrenal hormone Even if we can not prove that it is normally secreted into the blood, it appears highly probable to me that a substance present in the adrenocortical cells could be discharged into the circulation under certain conditions and could then be a factor in the production of 'diseases of adaptation" Presumably, mineralo corticoids do not circulate in the blood in high concentration under physiologic conditions, otherwise they would produce hypertensive disease Yet, perhaps with improved methods it will be possible to demonstrate mineralo corticoids in systemic blood

Apparently even typical gluco corticoids such as "compound F' or cortisone contain some mineralo corticoid potency as well The manifestations of the latter are largely dependent upon the available sodium in the blood hence it is possible that even endogenous gluco corticoids could under conditions of extreme sensitization for mineralo corticoid activity produce nephrosclerosis and hypertension in the human being In the rat this is certainly the case As I have also previously stated even cortisone causes marked hypertension and some nephrosclerosis in rats sensitized by unilateral nephrectomy and sodium In view of all these considerations it hardly affects the interpretation of the 'diseases of adaptation whether we do or do not accept desoxycorticosterone as a physiologic component of the adrenal cortex We used it in our early work merely as a test substance possessing high mineralo corticoid activity since other compounds with equal pharmacologic properties were not available

I think the enormous importance of conditioning factors' has not yet been fully appreciated by many investigators interested in

both of them, and the interaction between the two steroids was antagonistic It is not inconceivable to my mind that a deranged proportion between the gluco corticoids and mineralo corticoids secreted by the adrenal cortex may predispose to the formation of arthritis and other inflammatory changes in man as well, and that the treatment with cortisone and ACTH is effective because it rectifies a relative gluco corticoid deficiency

The very fact that on a sodium free diet even very large amounts of desoxycorticosterone produce no hypertension or hyalinosis shows that it is not the presence of the desoxycorticosterone molecule itself which causes damage It merely increases the susceptibility of the cell to local non specific stress

Dr Long brought up the apparent contradiction between the curative effects of ACTH and our production of collagen disease like changes with an impure pituitary preparation namely lyophilized anterior pituitary powder (LAP) In addition to ACTH this powder contains a number of unknown substances such as other pituitary hormones proteins, etc These contaminating substances apparently influence the effect of the ACTH contained in LAP We have actually produced arthritis with LAP and aggravated the course of formalin arthritis by pre treatment with this pituitary preparation Thus LAP acts somewhat like desoxycorticosterone while pure ACTH acts like cortisone

To support this view I might mention some recent experiments concerning the effect of pituitary and adreno cortical hormones upon an anaphylactoid reaction Rats are congenitally sensitive to egg white If you inject 2 cc of egg white intraperitoneally in the rat it develops an enormous swelling of the face and paws an 'anaphylactoid reaction' If just prior to the egg white injection the rat receives ACTH or cortisone, it does not respond Conversely, if the animal is pre treated with LAP or DOCA, the reaction to egg white appears to be actually aggravated Here again there exists some antagonism between LAP or DOCA on the one hand and ACTH or *cortisone on the other*

I do not think that we have any evidence to prove the existence of several corticotrophins but it looks as though certain factors present in LAP may so modify its effects that the end result is desoxy corticosterone like while pure ACTH produces cortisone like effects in the peripheral organs

The broader clinical and theoretical implications of all these findings have been discussed in more detail elsewhere (Selye H Stress Acta Endocrinologica Montreal (in press))

White What is the source of your LAP?

agent (local or systemic) Both mineralo corticoids and gluco corticoids appear to have an important effect upon such phenomena of membrane permeability Presumably, a change in the total quantity and relative proportion of these two types of steroids could help to combat the causation of "serous inflammation" by stress Conversely, a disproportionate excessive production of mineralo corticoids *not compensated by a commensurate secretion of gluco corticoids*, might aggravate the derangement in membrane permeability caused by a damaging agent

As regards arthritis, we performed a number of experiments based upon this concept As you will recall in rats given excessive doses of desoxycorticosterone, we frequently observed the appearance of an arthritis which in its acute stages imitated rheumatic and in the more chronic stages, a rheumatoid type of joint lesion Unfortunately we were never able to produce such joint changes with desoxycorticosterone consistently, but we noted that after adrenalectomy their incidence and severity increase We assumed that the adrenal cortex *might produce some antagonistic principle which in the intact animal* would tend to nullify this action of desoxycorticosterone These experiments have recently been confirmed by Pirozynski and Akert (*Pirozynski W and Akert, K Polyarthritis und Nebennieren* rindenhoromone *Schweiz Med Wchnchr 79 745 (1949)*) These investigators also felt that this increase in sensitivity is due to the removal from the system of antagonistic—presumably gluco corticoid—compounds

Recently we were able to show this even more clearly with the use of an improved technique for the production of arthritis We find that if a drop of dilute formaldehyde solution is injected into the vicinity of joints in the rate, it produces an inflammatory response in the periarticular tissues Pre treatment with desoxycorticosterone aggravates this 'formalin arthritis' and leads to excessive fibroplasia around the joint The resulting persistent granuloma interferes with the mobility of the joint and causes painful swelling histologically it resembles rheumatoid arthritis Curiously, cortisone pre treatment tends to inhibit formalin arthritis' In rats given both desoxycorticosterone and cortisone, the inhibitory action of the latter usually prevails and persistent fibroplasia cannot be produced In this respect, *ACTH acts like cortisone* presumably because it causes a predominantly gluco corticoid type of adrenal response Apparently, mineralo corticoids stimulate, while gluco corticoids inhibit, the *fibroplasia and edema formation in inflamed areas* Here the inflammation was not due to either of these two corticoids but the response to an irritating agent (formalin) was greatly influenced by

you describe and from beef they are rather imbalanced from the standpoint of trophic action They have very little adrenocortico trophic action, particularly if you make it up in neutral pH and saline The corticotrophic hormone has peculiar properties regarding its stability and I find it highly unstable at neutral, whereas it is quite stable at extremely low pH, so that I would feel that lyophilized anterior extract injected into the animal, might induce a state of relative insufficiency of the adrenal cortex and tend to have a marked trophic timulation on some of the other target organs A hormone imbalance would be produced characterized by a relative deficiency of cortical hormone

Long In this formaldehyde arthritis Dr Selye you said you get a granulomatous reaction In view of what Dr Loeb has said as to the reduction of granulation tissue or the prevention of its formation with cortisone you might anticipate if you injected cortisone into such animals that there would also be an effect on any granulation tissue that was present around the joints

Thorn Rats must be partially sensitive to beef anyway The thing which goes through my mind as part of this if you have a weak ACTH factor, it really does not stimulate the adrenal very much If you inject a second foreign protein before the effect of the first, the weak ACTH has worn off you would have a reaction to the second protein which would be much greater

Thorn To eggwhite

Selye That is the only one we know of

Thorn You are giving something else that is not causing a reaction but is making the second reaction greater Have you tried inactivation LAP so it has no physiologic effect?

Selye Yes We tried lyophilized liver tissue

Thorn Would inactivated pituitary produce the same effects?

Selye May I say in connection with Dr Sayers remarks that the corticotrophic effects as judged by the weight of adrenal cortex, were the same with LAP and the dose of ACTH which we used

Long You also have present thyrotrophic gonadotrophic and other pituitary hormones

Selye That is what we think influences the response

White Dr Selye in view of the quite opposing antagonistic actions that have been reported in relation to growth hormone and ACTH have you had the opportunity to try purified growth hormone as your possible LAP factor particularly since beef pituitary is exceedingly high in growth factor and low in ACTH?

Selye No Dr Ingle I think has something on this You have used purified preparations

Selye Cattle

Long Have you ever determined whether LAP effects occur in the absence of adrenal? Is this effect through the adrenal or is it due to something else?

Selye We have not studied the aggravation of arthritis after suprarenalectomy, but the effects upon the kidney and upon the blood pressure are inhibited by adrenalectomy, even in animals receiving maintenance doses of cortical extract That does not necessarily mean that the effect is entirely through the adrenal It may be dependent upon the simultaneous presence of adrenal tissue But since Dr Ingle has had some experience with this, he might want to comment on it

Ingle Three years ago, Dr B L Baker and I set out to determine the role of the adrenal cortex in causing the renal and cardiac changes seen following the administration of lyophilized anterior pituitary (LAP) In non adrenalectomized unilaterally nephrectomized rats on a high sodium, high protein intake, the administration of LAP caused pathologic changes in the heart and kidney as previously described by Dr Selye On the other hand, we have seen swollen joints in only one rat, and we have never seen periarteritis nodosa In confirmation of Dr Selye, we found that LAP did not cause heart and kidney damage in adrenally insufficient animals, but these experiments were complicated by poor survivals and by the reduction in food intake When we treated our adrenalectomized rats with adrenal cortex extract (ACE), keeping the intake uniform for both controls and experimentals we did obtain renal and cardiac damage in two adrenalectomized rats receiving LAP The intake of ACE was 8 cc per day in these rats, and it is possible that ACE itself was the damaging agent We started these experiments with the hypothesis that perhaps activation of the adrenal cortex is not the immediate cause of the damage following injection of LAP, but that the cortical hormones are essential to maintain the responsiveness of the animal We have not obtained a satisfactory answer to this question

Long May I ask what amounts of posterior lobe are included there in LAP?

Selye We tried to dissect the anterior lobe as carefully as one can That does not eliminate contamination completely The only thing I can say is that the posterior lobe is not the responsible agent because we could never copy the effects with posterior lobe even when lethal doses were given

Sayers We have worked with lyophilized anterior extracts which

every animal species that we could get The nephrosclerotic and hypertensive actions were first observed in the chick The chick is extraordinarily sensitive to collagen diseases of this kind I cannot remember exactly now but I think about 100 gamma of desoxycorticosterone per day is sufficient to kill a chick with acute malignant hyalinizing nephrosclerosis and edema Then we used the rat with the results which you know That was our most commonly employed laboratory animal not because it is most easy to produce hyalin diseases with it, but because it is a convenient animal for various surgical experiments We also found the same thing to occur (except for periarteritis nodosa) in the mouse, the cat, the dog and the Rhesus monkey

Long How about the joints? Have you produced such changes in any animal but the rat?

Selye Only in two out of all the monkeys we used did we obtain transitory painful joint swellings

At this time I would like to point out because apparently I did not make this clear in my paper although it was painfully clear to me, that you cannot get 100 percent arthritis under any condition whatever so it is not surprising that we did not get it more frequently in the monkey On the other hand we obtained arthritis quite frequently in the mouse In the dog we did not get any periarteritis nodosa or any myocardial nodules which are so easily produced by DOCA in other species

The dog responds with hypertension quite readily if sensitized by unilateral nephrectomy and sodium chloride It may even develop nephrosclerosis if large doses are given So essentially what comes out of comparative anatomic studies is that each species reacts slightly differently I think only the rat develops mesenteric periarteritis regularly The renal changes and hypertension are most constant and I know of no animal species that did not develop these As an addition to what I said about the anti arthritic and anti anaphylactic effects I should mention that during the alarm reaction (produced by bile duct ligature formaldehyde exposure to cold, surgical trauma, hemorrhage transection of the spinal cord) the animals are as resistant to formalin arthritis as they are with the highest doses of cortisone or ACTH and nearly equally as resistant to the anaphylactoid reaction caused by eggwhite I have a feeling that most of the so called non specific therapeutic effects which can be produced by various types of foreign proteins colloid metal suspensions fever therapy and other shock therapies may be largely dependent upon the endogenous production of ACTH

Thorn Did you ever have a chance to check arthritic rats

Ingle In agreement with the observations of Dr Selye, we find that LAP causes marked adrenal cortical hyperplasia in the non hypophysectomized rat, but has little effect upon adrenal size in the absence of the pituitary This suggests that the administration of LAP is a severe stress in itself and causes a pituitary adrenal response for this reason, whereas, it is actually low in ACTH content Remember that LAP is whole anterior lobe tissue and contains all of the pituitary hormones as well as other constituents of cells It is a mixture of foreign proteins The pituitary glands are heavily charged with bacteria when they come from the slaughterhouse LAP causes abscesses, as Dr Selye has described The various means of sterilization that we attempted were unsuccessful When we destroyed the bacteria, we also lost hormonal activity Finally, with the fine cooperation of Dr David Klein the glands were collected under as clean conditions as possible and were rinsed with acetone immediately LAP prepared from these glands was either sterile or had a very low bacterial count It no longer caused abscesses It caused little adrenal cortical hyperplasia in non hypophysectomized rats It no longer caused renal and myocardial damage Sterile LAP remained a potent growth stimulant, and it retained marked renotrophic activity It is possible that our handling of the glands caused a loss of some anterior pituitary hormone essential for the production of damage Now I do not believe that infection can account for all of the experimental production of "adaptation diseases" described by Dr Selye 11 Desoxycorticosterone acetate is the most potent agent in causing damage It has been reported from both Dr Selye s laboratory and from the Cleveland Clinic laboratories that alkaline extracts of anterior pituitary cause ' adaptation diseases We must still explain why non sterile LAP causes renal and myocardial damage in the presence of the adrenal cortices but not in their absence Moreover, we have some preliminary data which indicate that the drinking of large amounts of beef adrenal extracts causes heart and kidney damage in unilaterally nephrectomized immature female rats on a high sodium high protein diet Infection should not be a complicating factor in such experiments

Long May I ask one more question because Dr Fremont Smith suggested we might think of other experiments? Dr Selye, as I remember, most of your work has been done with the rat Has adequate attention been given to the dog for example? Presumably longer term experiments would be needed but the production of lesions in the dog would be a most convincing demonstration Have the effects of overdosage with DOCA been well studied in the dog?

Selye We have studied desoxy overdosage effect in practically

adrenalectomize rats there is a tremendous stimulus to hair growth (Butcher, E O *Am J Phys* 120 427 (*1937*)) We took *biopsies* of the skin starting 24 hours after adrenalectomy The rats we studied had been on a diet deficient in pantothenic acid for 30 days prior to adrenalectomy and as you know this causes atrophy of the hair apparatus and depletion of the melanin content of the hair bulbs Adrenalectomy acted as a stimulus to hair growth The effect was almost catastrophic, as every hair bearing follicle was stimulated and the deposition of melanin was increased The melanin was extractable and this was done by Dr Spoor working with us (Spoor, H J and Ralli E P *Endocrinology 35, 325* (*1944*))

Thorn We tried compound E on a hairy girl for a while after the reports in Science rubbing it on the skin and we could not affect the regrowth over a shaven area

Ralli They used DOCA

Ingle That supports the negative findings of two other clinical investigations, one at the University of Illinois Medical School and the other at Ann Arbor Baker and Whitaker tried cortical hormones on their own skins without noticeable effect

Conn I have a little preliminary clinical and laboratory information in this field which is as yet unpublished After having given 100 mg of ACTH to a normal man for a period of 15 days we were struck with what later appeared in the finger nails About three or four weeks after we stopped the ACTH this subject came down and showed me some defects in his finger nails I paid little attention to it but had him measure carefully the subsequent rate of growth of the nails By back titrating so to speak, we then realized that defects had occurred during the administration of ACTH So we took photographs of the nails and as the defects came to the ends of the nails we took them for cystine analysis Since that time we have watched carefully the nails of patients with Cushing s syndrome and have noticed the same type of defect in the nails of these patients We now have a large group of nails in the process of being analyzed

Another striking thing that I noticed in making general ward rounds was a patient who had had a *most* alarming stimulus She was a cardiac who had suddenly blown a whole lot of pulmonary emboli and was essentially moribund in oxygen for several weeks She recovered About four weeks later we noticed the same very deep defects in the nails These by measurement had occurred at the time of this alarming stimulus

For some time we have been interested in the possibility that enzyme systems requiring –SH groups might be importantly influenced by ACTH We felt that those proteins of the body known

that had been brought up in sterile colonies from birth? I am wondering how many of the manifestations, when we give toxic levels of some of these hormones, are due to other inhabitants that the animals harbor at that time How many rats would you need for the standard procedure in order to get enough arthritic animals?

Selye Without formalin just from DOCA sometimes 50 percent and sometimes you don't have any in a group of 20 animals

White The question of hair growth has come up, and I am interested in this purely from a scientific standpoint of course! But ACTH prevents the coming in of hair in denuded areas

Sayers Dwight Ingle ought to know about that

Long Would you like to say something about that, Dr Ingle?

Ingle Burt Baker and Wayne Whitaker in the Department of Anatomy at the University of *Michigan School of Medicine have* been working on this problem We have been collaborating with them First it was found that ACTH causes inhibition of hair growth in the rat Cortisone and 17 hydroxycorticosterone have the same *effect The local application of cortisone inhibits the growth of hair* at the site of its application Whole adrenal extracts also have this effect as does 11 desoxycorticosterone This observation is important The hormone can act directly on the tissue to which it is applied and does not have to operate through some central mechanism More recently we have extended these studies to an investigation of the role of the adrenal in the effect of estrogen upon hair growth in the rat The injection of estrogens causes inhibition of hair growth but if the adrenal cortices are removed the hair grows back despite the continued administration of the estrogen Since the adrenals enlarge and undoubtedly have increased secretory activity during the administration of estrogens it appeared that this increased production of cortical hormones mediated the action of estrogens upon hair growth However we have found that there is a dose of adrenal cortex extract which is not large enough to inhibit the growth of hair alone but does sustain the hair growth inhibiting effect of the estrogen in *adrenalectomized animals Here again is an example of the essen*tiality of the cortical hormone to maintain responsiveness although the change in secretory activity of the cortex was not required to cause the response

Ralli As a matter of record Williams (*Endocrinology 38, 368 (1946)*) applied estrogen to shaved areas of the skin of puppies and this inhibited the regrowth of fur The pictures are in the paper and are rather striking Dr Graef and I observed in the black rat (Ralli E P and Graef, I *Endocrinology* 32 *1 (1943)*) what Butcher had pointed out earlier in the white rat i e that when you

We found no trace of it in castrated male rats as judged by the growth of the accessory sex organs

Kendall Dr Mason has found that the daily administration of 100 mg of cortisone did not increase the excretion of 17 ketosteroids in a significant way In some patients the excretion of 17 ketosteroids decreased, in some there was no change and in others there was an increase of 2 to 3 mg

Ingle It causes atrophy of the testes in the normal male rat

Selye It causes a very marked atrophy of the ovary in the females

Bauer In light of Dr Loeb s remarks I wish to call attention to certain clinical observations It has been our impression that patients with severe rheumatoid arthritis, lupus erythematosus disseminatus and other mesenchymal tissue diseases are very prone to form keloids In several such patients on whom we have done successive biopsies for diagnostic purposes the formation of keloid has always resulted We have not as yet done any detailed studies on these patients

It is also of interest that Dr Cope, who has operated on most of the cases of Cushing s syndrome at the Massachusetts General Hospital has remarked on several occasions that wound healing in such patients is abnormal, always being much slower with the resulting scar thinner

We have not observed any gross abnormalities in wound healing in our one 12 day cortisone study, however in this instance the second biopsy was not done until a few days after discontinuing cortisone Our observations with wound healing have been confined largely to patients receiving ACTH To date we have observed no gross deviation from normal One patient with exfoliative psoriasis has been subjected to at least 15 skin biopsies Subsequent study of these skin biopsy scars would throw additional light on this problem Each time Dr Loeb talks about the effect of ACTH on wound healing I cannot help wondering what the outcome will be in one of our patients who is due to have a second knee biopsy done after 16 weeks of ACTH administration I would hate to think that because of poor wound healing sinus formation will result *

Loeb I have seen a patient one of Dr Perera's who has been on 200 mg of cortisone daily for three weeks She developed a furuncle on her chest which is completely indolent as far as granulation is concerned I don t think Dr Ragan has any evidence

* Editor s Note Subsequent communication from Dr Bauer—dated Jan 15 1950 states "The second biopsy scar appears essentially the same as the one done prior to ACTH therapy"

to be rich in cystine might be affected by a change in sulfhydril enzyme activity Such proteins are of course, insulin nails, hair, skin, etc We, therefore, began to study total sulfur balances and urinary excretions of cystine as affected by ACTH We found that there was an increase in total sulfur excretion under ACTH and that that total increase in sulfur could not be accounted for on the basis of the negative nitrogen balance associated with it In other words, there was a greater excretion of sulfur than could be accounted for by the negative nitrogen balance Cystine analysis by Sullivan's method shows a great increase in the excretion of cystine *Kinsell* has found a similar negative sulfur balance and an excessive amount of cystine excreted under ACTH stimulation Precisely what all *that means I am not ready to say, but I feel reasonably certain* that changes in hair, nails, skin and perhaps insulin are related to an abnormality in sulfur metabolism induced by ACTH

Thorn *Have you observed hair falling out in your patients under* long treatment and the hair on their heads becoming very thin?

Conn No

Thorn We have had some of those who looked like Cushing's, who have lost most of their head hair

Long How much greater is the negative sulfur balance than the negative nitrogen balance What was the ratio between the two?

Conn Urinary cystine may increase 75% to 100% above base line values while total nitrogen goes up only 30 to 50%

Thorn You felt that the renal threshold for amino acids was altered?

Conn I don't know

Ralli There was a symposium on amino acid excretion May 10 1948 at the N Y Academy of Sciences *Dr Pitts* do you remember those reports?

Pitts I did not go

White *What about the amino aciduria in Fanconi's syndrome?* Is this due to augmented excretion of all amino acids?

Bloch Methionine

Long And some peptides

Bauer In our two 12 day cortisone studies increased hair growth was observed in each patient

Thorn You get hair on the upper lip But some of the hair disappears from the head

Long You have not examined the excretion of other amino acids under these conditions?

Conn No

Selye What is known about the androgenic effects of cortisone?

STEROID METABOLISM IN THE ADRENAL CORTEX

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DR LONG encouraged me to talk about the biosynthesis of cholesterol This subject has no direct connection with the metabolism of adrenal steroids except for the fact that cholesterol itself may very well be the parent substance from which adrenal steroids are derived

The problem of the biosynthesis of cholesterol is in many respects an exercise in organic chemistry The problem at the present time is to find out what carbon precursors are involved and to find experi mental conditions in which the synthetic mechanism can be demon strated under relatively defined conditions As you all know choles terol is readily synthesized by animal cells You find a great variety of steroids throughout the living world but cholesterol occurs only in animal tissue There is one curious fact that one might mention namely that certain heterotrophes intestinal parasites and certain insects cannot synthesize steroids but rely on the host for the supply of steroids If such parasites are grown in synthetic media one has to add cholesterol as a vitamin as it were The one exception to the general occurrence of steroids as far as we know is that bacteria do not contain a compound which has the cyctopentanophenan threne structure so one has to assume that the steroids are not involved in cellular metabolism in a general sense but are compounds of special ized function

The early work on tracers by Schoenheimer and Rittenberg (9) made it seem very likely that the cholesterol structure was built from small metabolites in animal tissues This early suggestion was later on taken up by Rittenberg and myself A paper by Sonderhoff and Thomas (13) came to our attention in which the assimilation of deuterio acetate by yeast was studied It was found by the authors that the unsaponifiable fraction of the yeast which had been kept on deuterio acetate, as the only source contained a very large concen tration of deuterium (12) It thus appeared that there was a rela tively specific utilization of acetate for steroid synthesis Chemists

that there is any interference with epithelization by ACTH or cortisone

Thorn We had one patient with Addison's disease who always got keloids with pellet implantation Last year we implanted E for the first time instead of desoxy and no keloid formed In one patient with lupus we had to stop the ACTH because of the indurated, inflamed skin lesions They healed quite promptly after we stopped ACTH

Selye Do you have the impression that DCA actually aggravates this reaction?

Thorn I cannot say that

Selye Do you have the impression that DOCA actually aggravates effects of cortisone and ACTH I had a communication from Dr Sprague and he informs me that with large quantities of cortisone, 100 mg 200 mg per day, he did get a noticeable increase in hair growth in the female

Thorn We have observed that, and also acne The hair growth is in areas where you would expect an androgen to act

Sayers It is still a question whether it is androgen or some other degradation product of cortisone which is having the same effect upon that particular target cell as androgen

Long Perhaps we ought to proceed now to the fourth subject of these discussions which is 'Steroid metabolism in the adrenal cortex ', which will be opened by Konrad Bloch

acetic acid are utilized to the same extent For that purpose we prepared acetic acid which was labeled by C[illegible] in the methyl group and C[illegible] in the carboxyl group This material was fed to animals and the ratio of the two carbon isotopes was determined in the iso lated cholesterol We carried out several such experiments and the ratio of carbon atoms derived from methyl groups of acetic acid to the number of carbon atoms derived from carboxyl groups of acetic acid came out to be 1 27 This value represents the average of three experiments If one makes the assumption as a working hypothesis that all carbon atoms of cholesterol are derived from acetic acid—and as we shall see later there is some evidence to support this assumption—then this ratio would indicate that of the 27 carbon atoms in the cholesterol structure 15 are derived from methyl carbons and 12 from carboxyl carbons of acetate We have furthermore determined this same ratio also in some cholesterol degradation products The reaction which turned out to be very helpful in this respect described by Mauthner in 1896 (7) is the pyrolysis of cholesteryl chloride If cholesteryl chloride is heated to 350 then the molecule splits at carbon atom 17 and you obtain a mixture of aliphatic hydrocarbons iso octane and iso octene and a residual high boiling fraction This high boiling fraction seems to have the intact polycyclic structure and analyses for a hydrocarbon C[illegible] H[illegible] containing one double bond It still has the two methyl groups although the one angular methyl group has apparently mi grated to carbon 17 We have determined the relative utilization of methyl and carboxyl carbon of acetate in the nuclear fraction if I may call it that and in the aliphatic fraction of the cholesterol molecule and the ratio of methyl to carboxyl carbon in the nuclear fraction turned out to be 1 1 and the iso-octane fraction 1 50 There is without question a significant difference in these ratios for the two moities of cholesterol If it is assumed again that cholesterol is derived from the acetate only the experimental ratio of 1 50 comes closest to a ratio of 5/3 or five methyl carbons and three carboxyl carbons of acetate in the side chain of cholesterol For the nuclear portion the experimental ratio of 1 1 correspondents to a ratio of 10 methyl carbons to 9 carboxyl carbons

Our next problem was to determine the isotope concentration of individual carbon atoms of the steroid structure Few reactions of classical steroid chemistry lend themselves for this purpose and therefore progress in this direction has been slow Dr Little in our laboratory has converted cholesterol to cholestane and has carried out the chromic acid oxidation according to Windaus (16) to form allocholanic acid In this reaction carbon atoms 25 26 and 27 are

for many years have speculated on the mechanism of this biosynthesis They looked for similar structures which were available and could be presursors of a complex molecule of this type For example Windaus (16) suggested an appropriate folding of oleic acid to give rise to the polycyclic steroid structure There has been the suggestion by Robinson that an isoprenoid hydrocarbon such as squalene might be involved There has been no experimental evidence to substantiate these suggestions

We have investigated the role of a number of isotopically labeled compounds in steroid biosynthesis and have found that acetic acid is the most efficient source of hydrogen and carbon atoms for the cholesterol molecule (4) These early experiments were carried out by feeding labeled compounds such as acetate, carbohydrate intermediates, or amino acids to rats and examining the isotope concentration in the tissue cholesterol At that time deuterium was the only suitable tracer available and this technique has drawbacks because deuterium is not always an unequivocal labeling agent for carbon chains The advent of C_{14} made it possible to investigate the question of steroid precursors in greater detail The results obtained with this carbon isotope have confirmed the rather specific role of acetate in cholesterol synthesis Perhaps one should not overemphasize the specificity of acetate because it has been found in recent years that acetate is a building stone in a number of biosyntheses We know that the higher fatty acids are synthesized by what appears to be a multiple condensation of two carbon fractions, and that acetic acid participates in the synthesis of porphyrins and purines There are very few constituents of animal cells which do not use a two carbon fragment as a carbon source

Reichstein at one time, from purely structural considerations suggested that a three carbon compound might be an intermediate He suggested dihydroxyacetone If you divide up the C 27 structure of cholesterol you have nine times three and you can make a reasonable scheme on paper However we are quite convinced on the basis of our data that dihydroxyacetone or other intermediates in glycolysis are not involved

We felt that some information as to the mechanism might be gained by establishing which individual carbon atoms in the steroid molecule are derived from acetate and moreover which ones are derived from carboxyl groups and which ones from the methyl groups of the acetic acid In doing this we have availed ourselves of both the stable and the radioactive isotopes of carbon and have used both doubly and singly labeled acetate (6) One of the first experiments we did was to see whether the two carbon atoms of

carboxyl carbon atoms of this acetic acid The results here were quite clear as far as the methyl groups of the degradation product are concerned, namely, that the two angular methyl groups are derived from methyl groups of acetic acid and also that the tertiary carbon atom 10 to which the angular methyl carbon 18 is attached is derived from a carboxyl of acetate We have evidence however that the carbon atom to which the second methyl group is linked is not a carboxyl carbon of acetate

Loeb Is the first one from carboxyl?

Bloch Yes, but the second one apparently was not derived from a carboxyl carbon I mentioned before that the angular methyl carbon is believed to migrate to the 17 position during the thermolysis of cholesteryl chloride One of the carboxyl carbons of the acetic acid which is obtained on chromic acid oxidation therefore would represent C_{17} We have evidence that a methyl carbon of acetate is the presursor for this position I might mention that we have done one independent experiment to confirm the results of the chromic acid procedure If cholesterol is treated with a dehydrogenating agent such as palladium, then rings A and B become aromatic (11) As a result the angular methyl group 18 is squeezed out as it were in the form of methane yielding one mole of methane per mole of cholesterol By this procedure we have obtained methane from cholesterol, prepared either from carboxyl or methyl labeled acetic acid It contained isotopic carbon only when methyl labeled acetic acid was used confirming the results obtained by means of chromic acid degradation There is only one further point which should be mentioned in this connection I said before that in our opinion the majority of carbon atoms of cholesterol is derived from acetic acid The principal reason why we believe this to be so is the following If you prepare cholesterol from let us say carboxyl labeled acetic acid and you find a specific activity of 100 counts per minute in the total cholesterol then you will find that the isotope concentration of individual carbon atoms will be either about twice as much or 200 counts, or zero The same relationship is found if you use methyl labeled acetic acid If you assume on the other hand that only 50 percent of the carbon atoms of cholesterol are derived from acetate then you would expect that every individual carbon atom which is labeled should have an activity about four times that of the total cholesterol We feel that these quantitative relationships are the best evidence for the assumption that a very large part of the carbon atoms in cholesterol is derived from acetate The nature of the intermediates which are subsequently formed from acetate is completely obscure I might say that the available evidence

split off in the form of acetone The acetone can be further degraded to iodoform and this iodoform will represent the two methyl carbon atoms 26 and 27 of the cholesterol side chain From the analysis of iodoform and of the total acetone the carbonyl carbon (C_{25}) can be determined by difference If one prepares cholesterol from acetic acid in which the methyl group is labeled, then the iodoform derived from the acetone but not the carbonyl carbon will contain isotopic carbon Conversely, if one prepares cholesterol from acetate containing isotopic carbon in the carboxyl group, the isotopic carbon will be present primarily in the carboxyl group of acetone We must conclude then that C_{26} and C_{27} are derived from methyl groups of acetate and C_{25} from a carboxyl carbon We had hoped to carry out a stepwise degradation of allocholanic acid in order to analyze the remaining carbon atoms of the cholesterol side chain but the yield of allocholanic acid is so poor that this has not been feasible as yet If we inspect the iso octyl side chain of cholesterol with the knowledge that the two end methyl carbons are derived from methyl groups of acetic acid and the tertiary carbon from a carboxyl group of acetic acid and consider furthermore that the ratio of methyl to carboxyl carbons is 5 to 3, then we may suggest that carbon atoms 21, 22, 24, 26, and 27 are derived from methyl carbons and 20, 23 and 25 from carboxyl carbons of acetate We hope to establish the origin of carbon atoms 20 to 24 by direct analysis

We have only little information regarding the origin of individual carbon atoms in the nucleus We were particularly interested in the synthesis of the angular methyl groups and of the isopropyl group in the side chain of cholesterol There are very few branched chain compounds present in animal tissues and those which are present are essential, namely, the amino acids leucine and valine, and vitamin A It seems that the steroids are the only compounds with branched structures which the animal itself can synthesize For this reason we have tried to find some tie up between the essential amino acids, leucine and valine and the synthesis of cholesterol (2) I will return to this point later One method for isolation of the angular methyl groups of cholesterol was the following rather crude procedure but in this particular case it seems to have furnished the desired results According to the literature the treatment of any compound containing carbon bound methyl groups with chromic sulfuric acid mixtures under specified conditions yields acetic acid We have applied this degradation to the nuclear hydrocarbon $C_{1}H$ and we obtain about 1 5 moles of acetic acid per molecule of hydrocarbon This acetic acid was analyzed as such and was converted to iodoform by way of acetone to yield separately the methyl and

sciatic nerve, and liver The specific activity in cholesterol of liver was 10,000 In brain it was 50 in spinal cord it was zero and in sciatic nerve it was 12 We are very doubtful that these low values are significant It is difficult to remove the last traces of blood from these tissues and the small radioactivities in the cholesterol of nervous tissue may well be due to contamination by blood cholesterol These are adult rats *In young rats the story is different* As Waelsch and Sperry have shown, in young rats there is a deposition of cholesterol in the brain and this process seems to cease after myelinization We have here a unique case of an organic molecule in the animal body which in some tissues seems to be completely inert metabolically There has been evidence recently that at least one other organ can synthesize cholesterol and that is the adrenal cortex Chaikoff and his collaborators (14) have carried out very similar experiments namely incubating slices of adrenal cortex with labeled acetate and have found the formation of isotopic cholesterol in this tissue The rate of synthesis which can be calculated on the basis of Chaikoff's data is about 15 gamma of cholesterol per rat adrenal per hour A rat adrenal I suppose weighs about 40 mg Is that correct Dr Long or Dr Sayers?

Long It is a little high for small rats

Ingle It depends upon the sex of the animal

Sayers 20 to 50 mg

Long It is a little below that Dr Bloch

Bloch One can then recalculate for the beef adrenal in Dr Pincus experiment If you assume the beef adrenal to weigh 40 grams—would that be correct or more?

Pincus It would be less than that

Bloch Assuming that a beef adrenal weighs about 40 gms, you would have in one beef adrenal roughly 50 mg of cholesterol synthesized per hour I don t know whether this would be an adequate amount to account for the formation of the various corticoids The interrelationship of cholesterol and the cortical hormones has as you know been clearly brought out by the experiment of Sayers and Long and Doherty and White at Yale (6) who observed the very specific decline of adrenal cholesterol under the influence of ACTH a decline which was not paralleled by a decline of cholesterol in other tissues This loss of adrenal cholesterol is accompanied by the appearance of glycogenic activity I have calculated from the data of the Yale group the decline in milligrams in a three hour period It amounts to about 1 mg per adrenal in a rat of 200 gm weight I don t know whether this figure is of the same order as the

excludes the higher fatty acids as intermediates It is not a question of folding of the higher fatty acids

I mentioned before that the branched chain amino acids, leucine and valine look attractive as precursors of cholesterol and Dr Zabin in our laboratory has done some studies with a compound which is rather intimately related metabolically to leucine There is evidence that isovaleric acid is produced from leucine by oxidative deamination and decarboxylation We have labeled isovaleric acid in the methyl carbon atoms of the isopropyl group by C_{13} and the carboxyl atom by C_{14} This compound is utilized to a much larger extent for cholesterol synthesis than is acetic acid (17) Mole for mole this compound will afford about five times the isotope concentration in cholesterol as does acetate This is not true for the entire molecule It is true only for the isopropyl portion of the molecule The carboxyl moiety of isovaleric acid is not utilized to a greater degree than acetate We do not know whether this implicates isovaleric acid as an independent precursor or carbon source of the steroid structure We had suspected at first that this branched compound might be utilized for the isopropyl group of the cholesterol side chain or for the angular methyl groups and their adjoining carbon However since these positions can be derived from acetate it is clear that isovalerate cannot be an exclusive source of these configurations

I think this roughly summarizes our scanty knowledge on the precursors of cholesterol I might mention very briefly some of the biological conditions under which cholesterol synthesis occurs In our earlier work on cholesterol metabolism we tried to find a simpler experimental system than the intact animal and we observed that liver slices would synthesize cholesterol as measured by the incorporation of isotopic hydrogen or carbon at a rapid rate (3) When the amounts of cholesterol synthesized per unit of time and per unit of liver weight were calculated it was found that the rate of the *in vitro* reaction corresponded roughly to the rate observed in the intact animal A number of other organs, among them the intestine, testes kidney heart muscle in the form of slices were tested without any success and we therefore concluded that the liver probably was the major site of cholesterol synthesis One organ does certainly not participate in steroid synthesis namely the brain Under conditions allowing for rapid cholesterol synthesis in all other tissues the brain cholesterol is completely free of radioactivity We have repeated earlier experiments of Waelsch and Sperry (15) and have administered acetate of high C_{14} content to the rat and isolated the cholesterol from the brain spinal cord

to dehydroepiandrosterone, some pregnane derivatives such as Δ5 *pregnanediol -3 β 17 β-one-20 have been isolated by Hirschmann* and Hirschmann (5) from urines of adrenal tumor patients but there are only four or five such compounds with these structural features in rings A and B which are found among the multitude of urinary metabolites The excretion of dehydroepiandrosterone is particularly conspicuous in adrenal tumors as Mason and Kepler have shown (8)

Anker and I have done some experiments on the catabolism of cholesterol which may have some bearings on the subject under discussion (1) It has been an attractive idea for some time that cholestenone is the intermediate in the conversion of cholesterol to the excretory or saturated sterols (10) The formation of the isomeric saturated sterols is most readily explained by assuming the formation of an intermediate which contains a 4 5 double bond and a beta hydroxy group at 3 Schoenheimer, Rittenberg and Graff a number of years ago fed labeled cholestenone and demonstrated its conversion to coprosterol (12) We have more recently fed labeled cholestenone and have obtained labeled dihydrocholesterol Dihydrocholesterol accompanies cholesterol in the tissues and usually amounts to about 1 or 2 percent of the cholesterol found in various organs This conversion of cholestenone to dihydrocholesterol takes place in the tissues and not in the gut On the other hand the conversion of cholestenone to cholesterol does not seem to occur at an appreciable rate In our experiments in which we used labeled cholestenone we found very small quantities of deuterium in the tissue cholesterol and we therefore concluded that cholestenone is not an intermediate in the total synthesis and that the conversion of cholestenone to cholesterol is not readily reversible The inability of the tissues to reduce cholestenone back to the unsaturated alcohol may be of significance in interpreting the chemical nature of the urinary metabolites in their relation to the cortical hormones Merely by analogy one may perhaps reason that any Δ5-stenols in the urine are not end products but represent intermediates in the synthetic paths which for some reason or other are blocked or overworked This hypothesis may be put to a partial test in the following manner As I mentioned before cholesterol which is synthesized from labeled acetate contains as far as we know isotopic carbon in all positions particularly in those of the side chain If we assume that dehydroepiandrosterone is formed as an intermediate from cholesterol and if it is subsequently converted to pregnane derivatives or compounds with the ketol side chain it would mean the addition of two carbon

quantities of corticoids which could reasonably be produced under stimulation

Long That would be about 5 percent of its weight in the gland, as steroid?

Bloch This is based on the 4 percent weight

Ralli Is that on the rate of 100 mg of adrenal or on the absolute weight of tissue?

Bloch On the absolute weight I think your average weight data indicated between 35 to 40 mg and if you take 4 percent of that being cholesterol, even a 50 percent decline would amount to roughly 0 8 mg

Sayers This is a minimum figure you have here, that is just on the basis of storage of cholesterol It does not take into account the fact that the gland can synthesize cholesterol?

Bloch It does not make allowance for that

Sayers There is the possibility that the gland could contribute

Bloch It would be very interesting to see whether the rate of cholesterol synthesis in the adrenal is effected under the same conditions I would like to make a few speculative remarks on the conversion of cholesterol to the steroid hormones

We may start out with the thesis that cholesterol is the precursor of cortical hormones as it is for the bile acids and for at least one of the steroid hormones namely progesterone The question then arises what are the intermediate steps in the formation of corticoids For instance is the cholesterol side chain removed carbon by carbon with the intermediary formation of bile acids and at what stage is oxygen introduced into ring C? It seems unlikely that bile acids are intermediates in the formation of cortical hormones I don t know whether the adrenal has ever been examined for its content of bile acids Furthermore a very important fact, as Dr Pincus told us yesterday is that the oxygenation of carbon atom 11 can occur at a late stage after the entire side chain has been removed It is attractive to assume that the presence of a hydroxy group at position 12 will facilitate the biological introduction of oxygen at 11 in analogy to the organic chemical approach There is one compound among the multitude of adrenal metabolites which looks attractive as an intermediate not only for the formation of the cortical hormones but also for androgens and estrogens and that is dehydroepiandrosterone This suggestion is not news and has been advanced for instance by Hirschmann and Hirschmann (5) The characteristic feature of this compound is that it still has the β 3 hydroxy group and has the 5, 6 double bond as in cholesterol In addition

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DISCUSSION

Long Before we have a general discussion on Dr Bloch s paper I think Dr Conn has some material on serum cholesterol in relation to cortical hormones that might be of interest Would you like to talk about that now, Dr Conn?

Conn Yes About a year and a half ago we began to follow serum cholesterol values in all of our metabolic studies on subjects who were receiving ACTH We determined both the total and free cholesterol of serum by the Schoenheimer Sperry technique

Our working hypothesis was built around the following facts Dr Long and his associates had demonstrated in their acute experiments on rats that a sharp fall of adrenal cholesterol occurs when the animals are subjected to stress or when either epinephrine or ACTH are administered No significant decrease in the cholesterol content of other tissues was observed Although not proven it is generally assumed that the disappearance of adrenal cholesterol under these circumstances represents conversion to steroidal hormones Dr Bloch has shown that the liver is a major site of cholesterol synthesis It is an important source of serum cholesterol Chaikoff and his asso ciates have recently demonstrated *in vitro* synthesis of cholesterol by surviving adrenal cortical tissue Finally, the capacity of the cortex under stimulation to deliver large amounts of steroidal hormones into the blood is now clear

With these facts in mind the source of large amounts of cholesterol to satisfy continuing adrenal requirements under conditions of chronic and intense cortical stimulation became of considerable interest The fact that adrenal cholesterol falls sharply upon stimu lation of the cortex indicates that although the gland may be capable of synthesizing cholesterol this process either is not keeping pace with the rate at which cholesterol is being withdrawn or cholesterol

atoms Then such a pregnane derivative or cortical steroid derivative should have isotope in the ring structure, but not in the carbon side chain

Pincus Did you ever try that with pregnandiol that you isolated?

Bloch You cannot do that with deuterium because in the oxidative procedures which would have to be carried out deuterium would be largely lost

Pincus Could I interrupt to say that in some experiments on pregnenolone metabolism, the result that you would predict occurred in the sense that the only isolated metabolite was pregnandiol–3–α 20–α (Pearlman W H and Pincus G Metabolism of pregnenolone *Federation Proc* 5–No 1, 79 *(1946)*)

Bloch I don't know whether I am mistaken on that but the number of such 3–β hydroxy going to delta 5,6 compounds is small compared to the saturated hydroxy compounds and the 3 keto compounds

Pincus Pregnenolone as such has never been recovered from urine It has been recovered from pig testes, dehydroisoandrosterone is the only one

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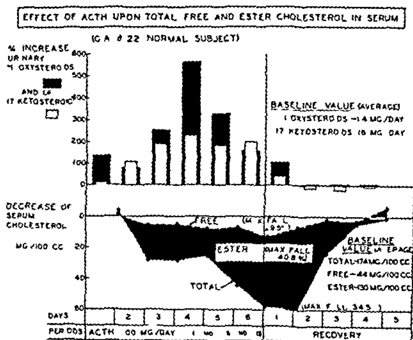


FIGURE 7

at the expense of the ester fraction Somewhere between the 4th and 6th days of stimulation there occurs a *tremendous* fall in the ester fraction accompanied by a much smaller fall of the free that is, the ester fell 78 mg (46% of 171 mg) as compared with a 16 mg fall of the free fraction (28% of 60 mg)

Upon cessation of ACTH a full three days is required for the ester fraction to approach its baseline value while the free fraction bounded above the baseline in 24 hours

Pincus How do you measure the urinary corticoid, by what method?

Conn We measure urinary corticoids by the formaldogenic response to periodic oxidation

Figure 7 represents the response of another normal subject It shows again the initial lowering of the ester fraction its sharp fall on the fifth day of ACTH, reaching a low point 30 hours after ACTH was stopped and then rising rapidly The free fraction again returns to baseline levels before the ester fraction Maximal falls in this case were 52 mg /100 cc for the ester and 13 mg /100 cc for the free fraction

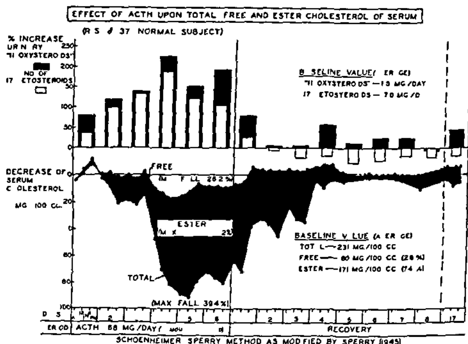


FIGURE 6

synthesis is inhibited The latter would seem to be an unlikely possibility

We, therefore followed the serum cholesterol of normal people, Addison s disease and one case of Cushing s syndrome before, during and after a 6 to 10 day period of administration of ACTH Only a mild depression of serum cholesterol occurs in normals during the first 2 or 3 days of ACTH On the third to fifth day total cholesterol falls precipitously and mainly at the expense of the ester fraction Figure 6 shows examples of these results The upper halves of these charts showing the percentage increases in urinary 11 oxysteroids and in 17 ketosteroids under ACTH stimulation are included to indicate that great physiological responses to stimulation have occurred The lower halves of the charts *show the decreases* of serum cholesterol in mg per hundred cc The ester fraction being the difference between the total and the free is represented as the black area between the two determined values Note that for 3 days the free cholesterol does not fall, but that by the middle of the second day the total cholesterol begins to fall all

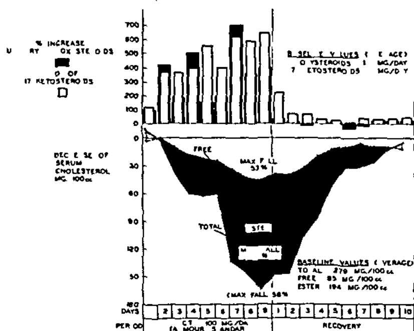


Figure 9

Long The corpus luteum is as high as the adrenal

Conn Yes About 90% of its cholesterol is esterified but again it is a producer of steroidal hormones It is tempting to think of the possibility that esterified cholesterol may be the immediate precursor of the steroidal hormones and that when the adrenal cortex is forced to produce at an excessive rate over a long period of time, the synthesis of cholesterol in the adrenal fails to keeps up with the need for it and the esterified cholesterol is then withdrawn from the circulating blood The other possibility of course is that some steroid which is produced under stimulation by ACTH is affecting hepatic esterification of cholesterol or the actual synthesis of choles terol In other words, there may be a specific pharmacological effect upon the liver of a steroid produced under ACTH Against that possibility is the fact that with 200 mg of compound E per day in normals, we have been unable to show a significant change

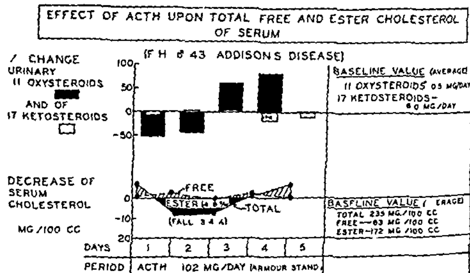


FIGURE 8

Figure 8 shows in contrast, the effect of 102 mg per day of ACTH in a case of Addison's disease where desoxycorticosterone had been stopped 10 days previously and the collection of baseline data begun 5 days previously Note that the free fraction didn t change at all, that the total fell only 3 5% and that it *rose* a similar amount even while ACTH was being continued These changes are not significant ones The 17 ketosteroids didn't change small changes in '11 oxy steroids occurred on either side of the baseline No significant metabolic changes were observed

Figure 9 shows the results obtained in a case of Cushing s syn drome with bilateral cortical hyperplasia given 100 mg a day of ACTH As you can see the serum cholesterol response was qualita tively the same as for the normals but quantatively much greater The maximal fall of the ester fraction was 115 mg /100 cc and that of the free 45 mg /100 cc Note, again, a delay of several days before the ester fraction falls sharply

Bauer In the Cushing s syndrome was the fall all at the expense of the ester?

Conn Mainly the ester What you ve seen is representative of the data The interpretation is another matter First of all it is to be recalled that adrenal cortical cholesterol is 90% esterified and that this is a unique situation with respect to other tissues of the body The closest—and correct me if I am wrong—is the liver in which about 50% of the cholesterol is in the ester form

EFFECT OF ACTH UPON SERUM CHOLESTEROL
AND URINARY STEROIDS
(R D ♀ 20 CUSHING S SYNDROME)

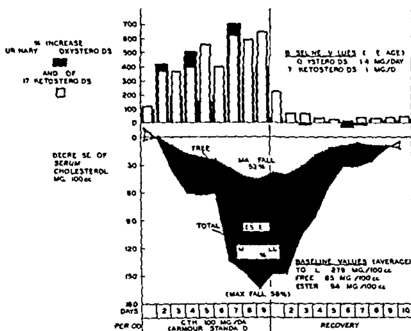


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in circulating serum cholesterol This holds true with cortisone in arthritis as well

Bauer What was that again?

Conn With 200 mg of compound E per day in normals or arthritics there is no decrease in circulating cholesterol This suggests that at this dosage level at least, compound E does not by a pharmacological means, depress production or esterification of cholesterol by the liver

White For how many days was the 200 mg daily dose continued?

Conn Ten days, the same length of time that we have used for the ACTH experiments

Bauer You did not carry any of your patients for weeks?

White Am I correct then in concluding that you have here a difference in the physiological action of ACTH and compound E?

Conn Well, yes and no It depends upon how one interprets our results *If one is thinking only in terms of peripheral pharmacological effects*, then 50 mg a day of ACTH does something which is not accomplished by 200 mg a day of cortisone If on the other hand, the fall of serum cholesterol produced by ACTH is due to its utilization by the adrenal for production of steroid hormones one would not expect compound E in any dosage to lower circulating cholesterol

White What I had in mind was an explanation which would be unrelated to the adrenal However such an explanation is no longer valid if cortisone will not do the same thing with respect to blood cholesterol that ACTH will do I have it clear that cortisone does not lower blood cholesterol?

Conn Correct at a dosage level of 200 mg a day

Pincus May I ask if the urinary cholesterol was measured after ACTH?

Conn No

Thorn 100 mg of compound E given daily to a patient with a nephrotic syndrome with a high blood cholesterol to begin with reduced cholesterol 150 mg percent and then it came back to the same level again after we stopped

White What is the explanation?

Thorn I don t know

Selye Desoxycorticosterone did not change the blood cholesterol level of normal rats In partially nephrectomized rats it produced a very marked drop in blood cholesterol

Thorn Our other stories are the same as Dr Conn's In patients with Addison s disease with roughly normal blood cholesterol administration of 100 mg of E caused no change in the serum cholesterol level

Conn There is another possibility to be considered That is the possibility of increased combustion for energy and of increased oxidation of cholesterol but if that were the case we should expect compound E to lower the cholesterol also

Pincus I think there is another possibility How did you measure this cholesterol? Determine the digitonin precipitatable steroid?

Conn Yes, by the Schoenheimer Sperry method

Pincus It would be very interesting in view of what Dr Bloch has talked about this morning to see whether there would be some ketonic substance, a cholestenone like substance What you depend upon in ordinary cholesterol determinations is ju t the presence of the 3–β–hydroxy group If that is oxidized to a 3-ketone you may have plenty of steroid there and miss it completely

You could extract the ketonic lipid from the total unsaponifiable portion and then try the Liebermann Burchard determination The typical color shows up pretty well with cholestenone This applies also to adrenal cholesterol determinations

Sayers I was just going to bring up a point I am unaware of any studies which have attempted the isolation of cholesterol from adrenal cortex We are dependent upon the Schoenheimer Sperry technique for the analysis of cholesterol The method employs digi tonin which precipitates steroids with the hydroxyl group in the 3–β position and the amount of cholesterol in the precipitate is deter mined by color development in the Lieberman Burchard reaction Is the method specific for cholesterol? I am unaware of any isola tion studies of cholesterol from the adrenal cortex

Kendall We have isolated a large part

Sayers Demonstrated it? This has not been published?

Kendall No

Pincus Marvin H Kuizenga mentioned that there is a large amount of cholesterol in his chapter entitled Isolation and Chem istry of the Adrenal Hormones in the book CHEMISTRY & PHYSIOLOGY OF HORMONES which the American Association for the Advancement of Science published in 1944 through the Science Press Washington D C

Sayers May I ask if it is demonstrated beyond doubt that it i cholesterol?

Kendall Yes It is demonstrated beyond doubt it is cholesterol

Sayers That satisfies me I am very happy to know it

Selye Dr Pincus was interested in urinary cholesterol

Pincus Suppose there is a low threshold for cholesterol

Long Look at the amounts involved I was going to say this fall in total blood cholesterol represents a total loss of cholesterol in the neighborhood of four grams

Conn That is over a ten-day period

in circulating serum cholesterol This holds true with cortisone in arthritis as well

Bauer What was that again?

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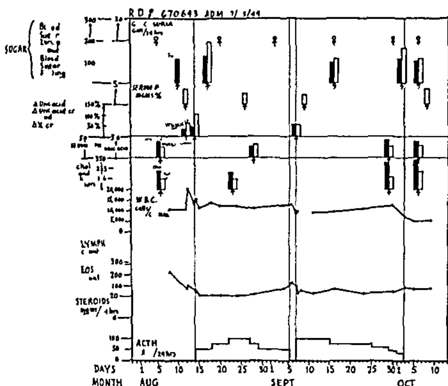
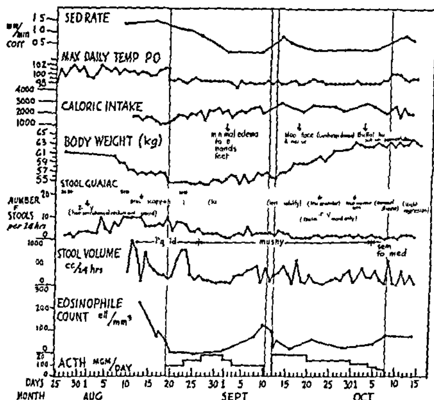


FIGURE 11

deficiency of androgen such as gynecomastia and loss of pubic hair I believe some excretion studies of the steroid hormones have been done but I am not sure It has been our experience in observing these patients over many years that one of the first evidences of improvement is a decrease in the amount of free cholesterol (Ralli et al, *Medicine* 28 301 (1949)) It is many months before this occurs but of all the serum constituents this appears to be the first to change in response to therapy

Bauer Our largest number of determinations were obtained on two patients with ulcerative colitis who were treated for approximately six weeks In the one case we observed very little change The other patient also had mild hepatic insufficiency as shown by 16 percent bromsulfalein retention and an alkaline phosphatase of about 13 units In this instance the total cholesterol and cholesterol esters which were low gradually rose to normal (Fig 10 and 11)

Conn After you stopped the ACTH?



RO 47yr ♀ Idiopathic Ulcerative Colitis 5+yrs Duration Last Exacerbation 3wks before entry Abdominal Cramps Nausea Vomiting Severe Watery Bloody Diarrhea PE 102-103 5° Wt loss, Toxic Lab Normal Except For Green Liquid Mucus Containing Stools – 3 to 4 + Guaiac, No Pathogens BA ENEMA Serrations and Loss Haustral Markings Proctoscopic Ex Severe I U C

FIGURE 10

Pincus There are urines which contain that amount of cholesterol as reported by Sobotka in certain cancer cases It is not impossible

Conn You may recall in Mason s paper in which ACTH was given to a normal individual that he found no increase in urinary cholesterol

Ralli In regard to the changes in serum cholesterol brought out by Dr Conn there is an interesting group of patients I refer to patients with severe cirrhosis of the liver The total serum choles terol is reduced and invariably the percent of free cholesterol is increased when the patients have had the disease for a long time and are emaciated These patients have clinical findings suggestive of a

some lowering of serum cholesterol occurred in the rat

White It happened in only one strain of rats

Long We did not emphasize this since the level of serum cholesterol is so low in the rat

Conn I would like to ask Dr Kendall and Dr Bloch a question a theoretical one in organic chemistry Is it true that blocking the reactive hydroxyl group on carbon three of free cholesterol such as we have in the ester form would make it easier for the adrenal to make substitutions on the other carbon atoms? That is if the adrenal were smart would it not choose esterified cholesterol as the precursor of steroidal hormones rather than free cholesterol? Would that be proper thinking Dr Bloch?

Kendall It sounds reasonable

Bloch There are quite a number of unspecific actions that happen with hydroxy groups and the conversion of ketones seems to be a very general reaction I wonder whether there is a possibility as Dr White has just mentioned of lipid storage I think the reticulo endothelial system has a great capacity to store or precipitate cholesterol and this has early been observed in the experimental atherosclerosis of rabbits We at one time injected a dog with labeled cholesterol We were much impressed by the very high concentration of this labeled cholesterol in the lungs and liver Of course this is exogenous cholesterol and not comparable to the endogenous cholesterol but I think the possibility might be considered that there is a withdrawal of cholesterol from the blood and some temporary storage in the tissues

Ralli Could I ask you a question? Does the liver lipid increase in the absence of the adrenals when you give ACTH and growth hormone?

White Yes with growth hormone

Ralli What about the distribution of phospholipids?

White We have not done this

Pincus Dr Conn, have you considered the possibility that this might indirectly be a thyroid effect?

Conn Yes It seems unlikely Addison s disease shows no fall in cholesterol on ACTH

Thorn It goes in the opposite direction

Conn Dr Thorn has shown that the basal metabolic rate falls under ACTH

Long Did the total lipid of the blood fall during this period?

Conn We don t know

Thorn The lipids don t fall in Addison s when given compound L

Bauer During the administration of it In this one patient with hepatic insufficiency both the cholesterol and cholesterol esters went up at the patient improved

Ralli Might that be a reflection of the nutritional state?

Bauer Yes

Conn Were ordinary urinary steroidal responses normal?

Bauer Yes

White I was going to ask Dr Selye whether in your wide variety of 'diseases of adaptation' you ever have seen any evidence of lipid storage diseases?

Selye No The only pertinent facts that I can think of are that the alarm reaction stimulates the phagocytic power of the reticulo endothelial system and hepatic fat deposition The latter especially in certain species such as the mouse and guinea pig It is markedly increased if there is some hepatic insufficiency (as after partial hepatectomy) in the rat That is a transitory storage which disappears after the animal enters into the resistant stage even if the stress is continued

Pincus Levin (Levin, L Studies of the endocrine mechanism involved in the mobilization of fat to the liver *Proc of the First International Congress of Biochemistry, 393 (1949)*) has described that as the direct effect of adrenal cortex action

Selye We have not seen fatty livers after cortisone

Pincus He used the mouse

White I had in mind the fact that in the mouse one can produce a marked increase in liver lipid with either ACTH or with the growth hormone Growth hormone produces liver lipid increase in the absence of adrenals so the question whether the ACTH effect on lipid is a growth hormone mediated factor is not yet settled I wonder whether the increased lipid of tissues and the decreased cholesterol of the blood could be due to increased rate of peripheral removal of these substances as a result of hormone administration Of course the failure to lower cholesterol esters of the blood with cortisone does not fit this concept This is the background of my inquiring of Dr Conn whether his findings with ACTH and blood cholesterol could be explained merely as an increased rate of peripheral removal of cholesterol

Long That is not true for sugar

White But for other substances Gordon has shown that it is true for thorium oxide Histologically you do open up the phagocytic bed with ACTH

Long You may recall that Dr White Dr Sayers and I reported in our first paper on the effect of ACTH on adrenal cholesterol that

same time went up They did not come down as fast as the cholesterol

Thorn Was there a loss of serum albumin during that period?

Kendall The albumin went up The globulin went down

Conn To increase the complexity of the problem, I would like to report upon another case of Addison s disease who, in addition has diabetes We gave ACTH and followed the serum cholesterol In this case no adrenals of course were present to make steroids nor were any increased amounts over the base line observed but the cholesterol went up very sharply as the diabetes became worse

Long The diabetes became worse with ACTH?

Conn Yes but this was probably due to cessation of in ulin during the experiment

Long It must have had some adrenal to stimulate

Conn There might have been a slight increase in 11-oxy but very mild

Ralli Could not that effect be mostly on the liver?

Conn Yes

White Did the diabetes get worse as measured by carbohydrate excretion or ketonuria?

Conn Glycosuria

Ralli Did you find in the cases to whom you gave ACTH and who developed diabetes, that the amount of insulin required for the control of the diabetes was proportional to the amount of ACTH given? Was there any evidence of antagonism between ACTH and insulin?

Conn Yes In normal people the diabetes produced with ACTH is very insulin resistant. It is resistant to 100 units of protamine zinc insulin in the normal individual who has diabetes produced by ACTH That is the diabetes is not responsive to 100 units of protamine zinc insulin per day

Long I would like to comment on what Dr Bloch said Chaikoff has shown that cholesterol synthesis in slices of beef adrenal from acetate occurs apparently without the presence of ACTH

Conn Without ACTH?

Bloch Yes

Long Apparently ACTH is not necessary for this synthesis We heard yesterday from Dr Pincus that ACTH is not necessary for 11-oxygenation to occur in the gland We seem perhaps to be getting a little closer at least in a negative way, to the point where ACTH is required for the secretory activity of the adrenal cortex

Sayers In that connection we have furnished evidence that ACTH

Long It would be interesting to know whether ACTH affects all the lipid constituents or only the cholesterol esters

White I was going to ask Dr Bloch if the next time he studies the rate of turnover of cholesterol in the various tissues they would do some of the larger blood vessels The question has always arisen whether cholesterol deposition in vessels is an inability to utilize what comes to it and, therefore, it piles up or whether it is an increased synthesis by that tissue *per se*

Bloch In the one experiment which I mentioned we compared the ratio of synthesis in liver, nerve tissues and in the aortae of rats The incorporation of labeled carbon into the cholesterol of these vessels was barely detectable It was somewhat higher than in the central nervous system but very much lower than all of the other organs I would say it was perhaps of the order of 1/50 that of liver and 1/10 that of muscle

White That means or could mean either low capacity to synthesize or low capacity to turn over

Bloch Yes In any case the metabolic regeneration, whether in the organ itself or by transport is very slow

Pincus I would like to ask Dr Bloch whether he made any studies of ovarian tissues because the same drop in cholesterol has been reported for ovaries with gonadotrophin

Bloch We have not checked that

Long Did you want to comment on the question Dr Conn raised about the effect of esterification in position 3?

Kendall It would be very nice if someone could show that from cholesterol right through to cortisone the gland did retain intermediate products through some such mechanism and that then it was released as cortisone I think some mechanism like that, however does exist but on the other hand in the medulla you have a situation which is hard to explain namely that adrenalin itself is present in the free form and does not seem to diffuse out under any condition but is released by some mechanism in paroxysmal sudden bursts of high blood pressure There is enough adrenalin in some cases of pheochromocytoma—and I have isolated a whole gram from one of those tumors—to kill the patient many times and yet it is controlled and is not held there as an ester

About this matter of cholesterol I was going to wait until it was all settled and then tell another result Dr Keith had a patient with nephrosis who was given cortisone After five days the cholesterol went from 300 to 700 mg per 100 cc and stayed there for some days and slowly came down The total fats in the blood at the

Ingle I might comment on some of our indirect information about the secretory capacity of the rat adrenal The capacity of these glands to secret hormone is very large but I have been repeatedly surprised by new evidence of its secretory capacity For example we set about to give enough beef adrenal extract to a rat to reproduce the metabolic effects (glycosuria) which were very easily caused by ACTH, and in order to do so we had to give up to 50 cc of ACE daily to the rat

Pincus How many grams of gland does 50 cc represent?

Ingle One cc represents 10 grams of beef adrenal

Ralli You give 50 cc to the rats a day?

Ingle Yes

Ralli How is it given?

Ingle It was given in the drinking water which is the most efficient way of administering ACE in the rat This was not the most impressive bit of evidence on the secretory capacity of the adrenals In order to get glycosuria in these rats we had to give only 1 mg of ACTH daily in 8 divided injections This caused considerable cortical hyperplasia A pair of adrenals usually did not weigh more than 80 to 100 mg and the largest pair weighed about 130 mg Giving ACTH by constant injection and in amounts up to 6 mg per day caused much more dramatic changes We have seen a single adrenal gland of 286 mg These animals became diabetic and lost weight so rapidly despite force feeding that they were at the point of death by three weeks I was much impressed by this evidence that the adrenal cortices can secrete enough hormones to bring the animal to death

White These were not hypophysectomized?

Ingle They were normal animals except that they were getting ACTH

Thorn What rate of infusion?

Ingle Six mg a day I forget whether it was contained in one or two cc given per day by constant injection

Fremont Smith Mature or young rats?

Ingle These rats were young rats but sexually mature They weighed around 200 gm initial weight

Thorn I think this illustrates the potentiality clinically where you are dealing with exogenous ACTH and bypassing the normal inhibitory mechanism The only good evidence that we have for an androgenic hormone being excreted as such from the normal adrenal other than a degradation product of another type of hormone has been the very large excretion of 17 ketosteroids following ACTH On the other hand 100 mg or 200 mg of compound E gave only

is not necessary for the synthesis of cholesterol Cholesterol concentration of the adrenal can increase following the removal of the pituitary and the same is true of adrenal ascorbic acid Apparently the accumulation of these materials in the gland, is not dependent upon the trophic hormone

Thorn The 'hypophysectomized' patient rarely develops very severe Addison s disease It is presumably the rate of hormone formation that is affected by ACTH

Ingle I would like to state a general question regarding the biological effects of ACTH This hormone has a very rapid effect upon the production of hormone by the adrenal cortex as Dr Pincus noted yesterday, and it also affects, at a slower rate, the growth and morphology of the gland How are these two effects related? We have been studying again the effect of ACTH upon the regeneration of the enucleated adrenal in the absence of the pituitary and in other conditions in which the enucleated gland does not ordinarily regenerate The long and short of it is that whereas we were previously unable to cause regeneration by the divided injection of ACTH we can now do so by giving continuous injections of ACTH

Long Growth hormone will enlarge the adrenal of the hypophysectomized animal according to the California group If you make the hypophysectomized animal grow with growth hormone, you also make the adrenal grow

Ingle Not very much

Long A fair amount according to their figures

Sayers Isn't that a secondary effect? Adrenal cortex is like other tissue in that growth hormone thyroid hormone etc are necessary for the maintenance of the functional capacity of the gland It seems to me that the effect of growth hormone is a secondary one

Regarding the effect of the trophic hormone on a target gland that is a very interesting problem for speculation Certainly in the case of adrenal cortex the changes which take place following the administration of trophic hormone are such that it is obvious that the chemical changes are very prompt and that the morphological changes come later Marked changes in cholesterol and ascorbic acid concentration of the gland occur in less than an hour whereas the morphological changes do not become visible for 12 to 24 hours

Conn There are some other points of dissociation as well The patient with Cushing s syndrome and bilaterally hyperplastic glands given a shot of ACTH put out a much greater amount of urinary steroidal products than in the base line indicating that although this gland is hyperplastic it is not receiving maximal functional stimulation

sections of some of these adrenals and there seems to be the so called persistence of the androgenic zone really the formation of an androgen at the site of a slowly involuting fetal cortex

They are real androgenic adrenal cases with very limited capacity to produce 11-oxysteroid and the androgen is not stimulated, at least by 48 hours of continued ACTH therapy so you have the capacity at some stage in the adrenal of forming a substance which is excreted as androgen unrelated to the 11 oxysteroid production

Bauer These patients present themselves for what reason?

Thorn Hair on the face and body, strong masculine development Quite different from the ordinary patient with adrenal insufficiency because they have this large amount of androgen

Fremont Smith In adolescents?

Thorn In adults and babies We have seen it in extremes in two children 1 month and a year old and in a woman at 80

Long I would like to ask Dr Bloch in the course of his studies when he fed this tagged acetate, if he had occasion to examine the ascorbic acid We are interested in the possibility that acetate is also the precursor of ascorbic acid in animals such as the rat which are able to synthesize their own

Bloch No

Long The technique would lend itself admirably to the solution of this question

Bloch I would like to ask Dr Pincus if the point of attack of ACTH might be somewhere between cholesterol and either C 19 or C 21 compounds If you assume that perhaps the dehydroepiandrosterone might be an intermediate you could explain an androgenic effect on this basis too by explaining that this intermediate could then either go to cortical compounds or androgens

Pincus The building up of side chains is a question which is completely a mystery as far as our data are concerned We looked very hopefully after perfusing with Δ4 androstenedione for C 21 compounds in the hope that maybe there would be a build up there but so far we have not found any That does not mean we may not On the other hand that compound is not dehydroepiandrosterone It may be that there is a series of sequential steps involved There may be enzyme systems that will only handle β-hydroxy compounds for building up side chains and will not handle and operate on unsaturated ketones So we have not given the final test that you suggest

Bloch You ought to test the ester of the dehydroepiandrosterone to protect the hydroxy group

Pincus We will do that

a small increase in 17 ketosteroid excretion I would be interested to know what the consensus of the group is concerning the studies that Dr Ingle reported, we may be just increasing the output of hormone in terms of grams of compound E like substance per day and the *androgenic excretory products observed may be degradation prod* ucts of some other steroid not originally a cortical androgen

Ingle We have a little evidence that relates to that In our work on ACTH and cortisone, we have seen regression of the testes and shrinking of the seminal vesicles Recently when we came to give ACTH by constant injection to hypophysectomized rats, we have seen partial maintenance of testis weight and fairly large seminal vesicles as compared *to similar animals without ACTH That sug* gested that the ACTH either contained small amounts of gonado trophic hormone or that the adrenals secreted some androgenic sub stance Gordon at California has recently found evidence that ACTH stimulated the seminal vesicles of immature rats I cannot remember whether or not they were castrated

White Has this been confirmed? At one of the Laurentian Hor mone Conferences, it was reported that this effect was not obtained with purified ACTH

Ingle Li failed to confirm the findings of Moon and Davidson with pure ACTH, but Gordon has reported confirmation

Pincus In connection with this I might mention Fieser s discus sion in wh ch he suggested purely on theoretical grounds, that the scission of the side chain of cortisone would give androsterone The latter has been isolated from adrenal tissue and is certainly an andro genic substance The order of magnitude of androgenicity is high so one might observe quite good effects If that were the case one would expect to find in human urine after the administration of ACTH, increased amounts of some 11 oxygenated metabolite of adrenosterone You remember at the Chicago meeting that Dobriner remarked that there was an increase in at least one case that he analyzed and if you looked at his figures closely you would see *the increase was rather disproportionate compared to other compo* nents It is barely possible then that the androgenic effect might be attributed to a substance like adrenosterone That requires proof It is a very interesting suggestion

Thorn We see a number of patients from time to time who have relatively high normal 17 ketosteroid excretion and yet have evidence of adrenal cortical deficiency The interesting thing with them is that their 17 ketosteroids do not increase under ACTH stimulation That has been observed in a male and a *female child* We have

by chromatography They find that the major fraction of the steroidal material is 17 hydroxycorticosterone and that there is possibly a small amount of corticosterone They cannot find any other steroid material They cannot find any material that takes the same position in the column as does desoxycorticosterone

Bloch Under what conditions?

Sayers 17 Hydroxycorticosterone is compound F That accounts for the major portion of the steroid material

Loeb Also a small amount of B?

Sayers A small amount of B but they are not certain of it

Loeb In dogs?

Sayers In dogs, yes

Bloch After ACTH?

Sayers No They are studying the action of ACTH Increased rates of secretion of hormone occurs under the influence of ACTH Of course there is the interesting question as to whether ACTH induces a qualitative change in the type of steroids elaborated

Loeb Just as a die hard Dr Long I would like to say if there is something of the nature of desoxy liberated one would expect to find an incredibly small amount in contrast to something like F or F by virtue of the fact that its salt and water effect is many times greater than that of the 11 oxygen or 17 hydroxy steroids Is that fair?

Long Chromatography will detect small amounts There was a paper given at the Academy of Sciences at Rochester reporting the use of chromatography for the separation of cortical hormones in body fluids

Pincus That is different Samuels is using paper chromatography

Sayers This is the column

Pincus Zaffaroni and collaborators use the column

Ingle I would like to recall some work that we did a number of years ago in which we set out to determine the kind of mixture of adrenal sterols required to sustain an adrenalectomized animal as well as his own glands We used work performance of the adrenalectomized rat as a criterion of efficacy of replacement therapy We did not get very far with this problem but we covered a good dose response range with cortisone alone and with a mixture of cortisone and desoxycorticosterone half and half When treating adrenalectomized rats for periods up to 14 days we were never able to sustain their ability to work more than about 60 percent of normal It would have been interesting and important to have added other compounds to that mixture I would especially like to know what corticosterone would do For this and other reasons I am convinced that

Long You ought to put the ACTH in that particular system

Pincus You are absolutely right

Bloch Another point, Dr Pincus presented evidence that the pregnane type of compound can be converted into an adrenal type compound with the ketol side chain It is very interesting if true because I don't think there is any analogous biochemical agent which can oxidize methyl to a carbinol

Pincus How do you account for strophanthidin? Aren't there steroids that have methoxy instead of the angular methyl group at C 10?

Bloch Perhaps plants can do it

Pincus I can offer a little suggestion for Dr Long I don t know whether the data are too significant, but recently Jensen and collaborators published a paper on the concentration of adrenal cholesterol after the administration of glucose and they found some increase (Steeples, G L, Jr and Jensen H Effect of blood glucose level on the secretion of adrenal cortex *Am J Physiol 157 418 (1949)*) We have repeated those experiments and find to our great interest that while the cholesterol increases the ascorbic acid decreases and this might indicate something about which I wanted to ask Dr Bloch that is is it possible that this administered glucose might be broken down rapidly enough to fragments which could then be resynthesized to cholesterol?

Bloch In our experience products of fatty acid oxidation rather than of carbohydrate breakdown are used as precursors in steroid synthesis Labeled pyruvic acid is a much poorer source for cholesterol than is acetate The question is still open to what extent pyruvate can be converted to two carbon compounds and whether the product is identical with that formed from acetate

Pincus What about acetoacetic acid?

Bloch Acetoacetate is probably formed in a side reaction whenever the oxidation of two carbon unites is interfered with

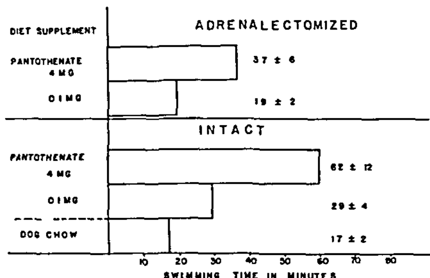
Long We have not talked very much about ascorbic acid I would like to hear any ideas as to the role of ascorbic acid in the secretory function of the adrenal cortex

Thorn I still would like to know how the group feels? Is there one hormone secreted under normal circumstances or are there two or three?

Long Are you a unitarian?

Thorn I change from day to day

Sayers Dr Samuels and Dr Nelson at Utah have been analyzing adrenal vein blood of dogs and fractionating the steroid material



EFFECT OF PANTOTHENIC ACID ON ABILITY OF INTACT AND ADRENALECTOMIZED RATS TO SUSTAIN SWIMMING

FIGURE 12

the adrenalectomized rats Apparently this vitamin can increase the rat s ability to withstand stress of this kind

Kendall Isn t there a limit to the capacity of the rat to swim?

Ralli If the rats are not removed from the tank promptly when they become exhausted they will die We have repeated the experiments at different seasons of the year and on a large number of rats The water is kept at a constant temperature

Ingle How long were the adrenalectomized rats given pantothenic acid before the test?

Ralli All the rats received the experimental diets for about 40 days This means that they received the pantothenic acid for that length of time

White Dr Ralli it was not clear to me whether these animals were on a pantothenic acid diet prior to adrenalectomy

Ralli Prior to adrenalectomy the rats received a diet containing 0 1 mg of pantothenate per 10 gm of food Following adrenalectomy the rats received as indicated in the chart either 4 mg of pantothenate daily or were continued on the 0 1 mg daily Intact

cortisone alone cannot be the only secretory product of the adrenal I worked with a little of Dr Kendall's amorphous fraction It would be very important to get back to isolating the active principle contained in the amorphous fraction Everyone has been distracted away from this problem

Thorn May I make a comment on that? I hoped the amorphous fraction was dead! When you give compound E in a standard dose to an adrenalectomized animal if you exceed his daily requirement you would in all probability have a continued loss of potassium If your test for function is a muscle test it appears that one argument against the quantitative aspects of substitution would be a continued depletion of potassium throughout your experiment

Loeb Are these animals eating?

Ingle Yes

Thorn Despite an adequate food intake one may occasionally observe a continued negative potassium balance with large doses of cortisone or ACTH

Ralli I wonder if I might bring up a point that I was going to speak of this afternoon but which seems appropriate now It concerns the ability of the adrenalectomized rat to work We have subjected adrenalectomized rats to the test of swimming in cold water, 18° C and measured their capacity to swim to the point of exhaustion All animals were tested after the experiment for the completeness of adrenalectomy by withdrawal of salt Different animals were used for each swim in order to rule out any possibility of adaptation to this form of stress The experimental diets were adequate in all nutritional substances and differed only in the pantothenate content which was either 0 1 or 4 mg per 10 gm of food Intact rats on identical diets were also tested and one group of intact rats from our colony that had been on the dog chow diet which is satisfactory for growth and development was tested Dog chow contains about 0 1 mg of pantothenic acid per 15 gm of food Figure 12 illustrates the results The intact rats on the experimental diet containing 0 1 mg of pantothenate swam for 29 ± 4 minutes as compared to 17 ± 2 for the rats on the stock diet When the pantothenate content of the experimental diet was increased to 4 mg per 100 gm of diet the intact rats more than doubled their swimming time The adrenalectomized rats on the experimental diet low in pantothenate swam 19 ± 2 minutes and on the high pantothenate supplement swam 37 ± 6 minutes The tests were done about 40 days after adrenalectomy The animals never received any hormone therapy Pantothenic acid increased the performance of both the intact and

Ingle No I have seen one adrenal gland which weighed over 600 mg and occurred in a rat given large doses of estrogens for many months It was simply full of cysts and contained no recognizable cortical tissue It occurred during some studies supervised by Dr Drips at the Mayo Institute

Long The large adrenals produced by prolonged administration of estrogens are usually depleted of their lipid

Ingle I assume that that was true of these large adrenals They were quite red in color

Selye I think the interpretation of the experiment depends very much on the adrenal structure Perhaps the animal died from hypercorticoidism perhaps from cortical insufficiency (because the adrenal became hemorrhagic and infarcted) Dr Floyd Skelton showed that animals kept on pantothenic acid deficiency—if they are not infected and if they are otherwise in very good condition—rarely develop any hemorrhages but if you expose them to any kind of stress their adrenals become very hemorrhagic and the animals die with some thing that greatly resembles the Waterhouse Friderichsen syndrome I think until we learn more about the structure of the adrenals it will be very difficult to say whether here death was due to hyper or hypocorticalism

Ingle The animals had glycosuria and were in strongly negative nitrogen balance This I think, could be explained only by assuming hypersecretion of hormones by these glands

Selye The glycosuria persisted until death?

Ingle I am not sure

Selye Obviously there would be some temporary hypercorticoidism I am just questioning the cause of death

Conn Dr Long I think the question that Dr Loeb brought up about his die hard position with respect to desoxycorticosterone or some salt retaining factor produced by the adrenal which of course is the important thing from a clinical point of view, must not be overlooked I would like to get on his side of the fence in this regard

Conn In panhypopituitarism with almost negligible urinary steroid excretions of 11 oxys and 17 ketos there may be very little disturbance in electrolyte metabolism In order to get an electrolyte effect from compound E in such a patient one has to give a pretty large dose and therefore one comes inevitably to the conclusion that the remnants of the atrophic adrenals if they are able to function a little bit must be putting out something in small amounts which is able to manage the electrolyte metabolism

Long Dr Greep and Dr Deane and their colleagues have made the statement that the water and the electrolyte metabolism of the

rats were treated in the same way that is they were either given the 4 mg pantothenate intake daily or continued on the 0 1 mg intake at the time when the adrenalectomized rats were operated on

Ingle Do you weight the rats when they swim?

Ralli No

Ingle Do you shave the rats?

Ralli No The adrenalectomized rats were shaved at the time of operation, but by the time of the swim test 40 days after adrenalectomy, the fur has regrown

Ingle Have these rats been depleted of pantothenic acid before adrenalectomy?

Ralli Not this group

Selye If this is a muscle efficiency test why use cold water?

Ralli We found that unless the water was cold the intact rats swam for such long periods that one had to spend most of the day watching them At 18° C swimming was sharply curtailed in the rats on the low pantothenate intake and in the rats on the high pantothenate intake *the swimming time was limited to a reasonable period of time*

Bauer Would not a nerve muscle preparation in the intact animal be a better method to test muscle exhaustion?

Ralli *I don't know*

Bauer This preparation offers one an excellent opportunity to study muscle function in adrenalectomized animals

Ralli *Our test is not hard to follow* The animals are pretty well exhausted when removed from the water

Ingle I was going to comment that your swimming times for *normal rats were much different than those of ours* but I think temperature explains the difference We used much warmer water and as you said—

Ralli They will swim *much longer*

Ingle Yes they swam all day

Ralli It became a matter of running the experiments satisfactorily *Dr Dumm does all of these experiments with the assistance* of one of the laboratory technicians

Kendall I would like to ask Dr Ingle about the architecture of *the gland that was stimulated with large amounts of ACTH* Does it look like an adrenal?

Ingle The outer portion of the cortex represented good viable tissue but *there was a great deal of hemorrhage and necrosis* in the inner cortex and medulla of each of these glands Dr C V Weller, Department of Pathology, University of Michigan, has described *these glands as representing vascular congestion*

Kendall You did not run the cholesterol ascorbic acid content?

Ingle No I have seen one adrenal gland which weighed over 600 mg and occurred in a rat given large doses of estrogens for many months It was simply full of cysts and contained no recognizable cortical tissue It occurred during some studies supervised by Dr Drips at the Mayo Institute

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Long Dr Greep and Dr Deane and their colleagues have made the statement that the water and the electrolyte metabolism of the

hypophysectomized animal are normal I think that is very far from the case

Conn I agree they are not normal On the basis of the sweat test we have found that there appears to be a deficiency of desoxy corticosterone in panhypopituitarism but that the deficiency is not the same as one finds in Addison's disease

Long Dr Pincus perfusion experiments show that there is a basal level of secretion that is independent of ACTH Isn't that quite in keeping with what we understand of the action of the hormone that it does not start anything new but merely accelerates the intrinsic metabolic processes Even in glands without ACTH stimulation there is a basal secretion going on It does not answer your point perhaps as to whether this trickle of secretion is sufficient to maintain water and salt metabolism That is why I mentioned that the water and salt metabolism is not normal It will maintain normal salt and water metabolism under basal conditions of the rat but if you put the animal up against conditions where it has to regulate the water and salt, it shows a failure to do so in the absence of the pituitary

Conn These patients with panhypopituitarism and low excretions, have hypoglycemia and show all evidence of deficiency of those fractions which take care of carbohydrate metabolism but their electrolyte metabolism also appears upset

Long They show hypoglycemia when fasting but if placed under conditions where more hormone is required for the regulation of water and electrolytes, they also show deficiency

Ralli We have done water tolerance tests on adrenalectomized rats and they certainly cannot handle water loads You can kill them by giving them 5 percent of their body weight in water

Sayers You mean hypophysectomized?

Ralli Either hypophysectomized or adrenalectomized rats

Loeb You have got the problem in panhypopituitarism of an associated thyroid deficiency which will also cause a marked delay in water excretion I wonder if one could find an assay method for salt and water hormone—if we knew approximately how much 11 oxy steroid was coming out of the effluent blood from the adrenal vein and then compare that effect in terms of desoxy it might perhaps give some useful quantitative information

Pincus There is a method available which I described very briefly that is sensitive to about two micrograms of desoxycorticosterone

Long A color reaction?

Pincus Essentially the Dorfman sodium retention test in rats We have used it with rats and found it quite useful

Loeb I think there may be a bioassay method which is more reliable I understand the Dorfman test has pretty wide variations

Pincus We have unpublished data If you set the animals up properly you can get good quantitative assays The difficulty always in dealing with adrenalectomized rats particularly in a balance study of that sort, is that you have to control conditions very carefully, the previous amount of ingested salt water and so on I think it will be a practical method I would like to say one thing again on the Conn side

Loeb You mean pro Conn?

Pincus Pro Conn or pro Loeb These are indirect data We had a group of schizophrenic patients who showed fairly good quantitative response to ACTH and stress and we compared them to a group of normal controls One of the things we found is that the output of corticoid substance was similar in the two groups the output of 17 ketosteroids was a little less in response to stress or ACTH a little but perhaps not significant We compared the output of urinary uric acid potassium and sodium and the lymphocyte change in stress The schizophrenic reaction in these respects was considerably less There are two possibilities that an inadequate amount of ACTH reaches the end organs for production of typical responses or there may be inactive presursor produced by the gland of the schizophrenic As I mentioned we have some evidence this may be so since there is a difference in the paper chromatograms in ketosteroids and one substance at least is missing I think there is the possibility that a lot of these so called hormonally inactive substances that Reichstein isolated which have been characterized very well chemically may be secretory products in an abnormal gland I think that should not be overlooked

Kendall This mention of the many compounds that Reichstein isolated raises the question whether they were in the gland As far as the important ones are concerned I have no doubt that they are, but when you come to the triol it seems to me very possible that both isomers could have been produced after isolation I understand that someone said that the odor in that laboratory was terrific when he was working on these glands It may be that some minor changes such as this could have been produced then by bacterial action Then there is one other point There is only one that has an alpha hydroxy at 3 It is possible that this also might have been produced by bacterial action

Pincus Isn t there any evidence for the 3 β hydroxy compound?

Kendall They are all beta

Pincus Those would be hormonally inactive?

Kendall They are, yes

Pincus If the gland for some reason or other produced that sort of substance rather than an $\alpha\beta$ unsaturated ketone you might have a 17 ketosterone precursor You would have a corticoid substance and still not have much hormonal activity

Kendall Zero

THE RELATIONS OF VITAMINS TO THE ADRENAL CORTEX

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IF I may take a minute or two I should like to make a few introductory remarks I have been through the literature and one interesting point is that the vitamins associated with adrenal cortical function are almost exclusively the water soluble vitamins I may have missed some references but I was able to find nothing significant in this regard I should be glad if anyone would correct this It seems fair to say that Morgan s work (18) stimulated interest on the relation of vitamins to the adrenal cortex If, however one searches through the literature it is apparent that the relation of vitamins to adrenal cortical function was suggested before Morgan s work was published In 1934 Bessey (2) reported that in ascorbic acid deficiency in the guinea pig the most outstanding changes were in the adrenal cortex I shall describe these more fully later

Ascorbic acid has probably been associated with the adrenal cortex more than any of the other water soluble vitamins The ascorbic acid content of the adrenal has been reported by Long (16) I might add Dr Long that you really should be giving this section because I am quoting you so often that you would have saved me a lot of work if you had just gotten up and given it yourself! The ascorbic acid concentration of the adrenal gland varies in different species of animals In rats and guinea pigs the concentration of ascorbic acid is approximately 400 mg per 100 gm of tissue It is well known now that the content of ascorbic acid in the gland changes when the animal is stressed (26 27) This is true with many forms of stress such as hemorrhage, exposure to cold epinephrine and thyroid administration Recently Wallach and Reineke (29) reported that feeding thyroxin reduced adrenal ascorbic acid to minimal values after 4 days This was followed by an increase in ascorbic acid which reached a maximum at four weeks and then leveled off Perhaps Dr Long would care to comment on this

Long Dr Sayers is the one because he reported that in chronic administration

White The lymphocyte response also tends to change as one

shifts from single administrations to chronic administrations of thyroid

Ralli As I said before, Bessey (2) showed that ascorbic acid disappears from the adrenals of guinea pigs maintained on a scorbutic diet Long (16), in studying guinea pigs, had remarked that the ascorbic content of the adrenal varies but if the pigs are placed on *a scorbutic diet, and receive 20 mg* of *ascorbic acid by injection* daily a constant value is attained Under such conditions if the vitamin is not given for 24 hours the adrenal ascorbic acid is lowered This would lead one to believe that the intake of ascorbic acid bears some relation to its concentration *in the adrenal* cortex Perhaps this is not a justifiable statement It is perhaps better to say that an absolute deficiency of ascorbic acid is associated with a decrease of this vitamin in the adrenal cortex The distribution of ascorbic acid in the adrenal cortex has been studied by several observers most recently by Greep and Deane (15) Using cytochemical methods they showed that in the normal adrenal a precipitate of silver occurs in all of the cells These are distributed as fine granules in the zona glomerulosa and as coarse ones in the fascicular and reticular zones It is generally reported that there is less ascorbic acid in the glomerular zones The impression one gets is that ascorbic acid is important to the adrenal cortex Whether it serves as a precursor of the hormone or not I would not venture to say I do *not know* of *anyone who has confirmed* Lowenstein s work (17) in this respect Has that ever been confirmed?

Long Not that I am aware of

Ralli It was published merely as an abstract of Endocrinology I found no further reference to it

Fractions of the vitamin B complex were the next group of vitamins studied in relation to the adrenal cortex Morgan (18) in 1939 observed that in the absence of the filtrate factors in the diet certain definite changes occurred in the adrenal cortex in rats *These observations were confirmed by Daft and Sebrell (5)* and others (1 6 19 25) One of the findings that impressed me was that in the deficiency states of these water soluble vitamins and I am referring particularly to pantothenic acid deficiency the changes in the adrenal cortex vary in degree under the same experimental conditions It was also interesting that Bessey in 1934 (2) reported disappearance of cortical fat staining material disappearance of doubly refractive cholesterol cortical hemorrhage and marked differences in staining and loss of vitamin C in the adrenal cortex *of guinea pigs on scorbutic diets* In fact Bessey stresses the fact that the most characteristic lesion found

in the scorbutic guinea pig was depletion of fat and ascorbic acid from the adrenal In reading and studying the effects of pantothenic acid deficiency on the adrenal cortex the type of change that occurs is similar to what Bessey described for a deficiency of ascorbic acid The differences are more in the degree the pathology follows the same pattern In a study that we did with Dr Graef (22), the adrenals of 284 rats of the Long Evans strain on diets deficient in the filtrate factor were studied There was considerable variation in the adrenal pathology The animals were kept on the diets for varying periods and sacrificed at intervals from 60 to 120 day Lipid depletion was the most consistent change although it was not present to the same extent in all of the animals We did not find more than 10 percent of the animals with hemorrhage of the adrenals and I think that that is probably because the animals were not stressed I think it was Dr Ingle this morning who was remarking that stress provoked hemorrhage in these deficient animals That may account for this wide variation in hemorrhage and necrosis in the adrenal cortex

Ingle It was Dr Selye

Ralli Studies have been done on thiamine riboflavin and pyridoxine deficient diets by Deane et al (9) Biotin incidentally was not included in the original first diet but that was corrected in later experiments These workers felt and I think this is still their feeling that these deficiency states are a form of stress and the results are probably due to the fact that this stress has stimulated the secretion of ACTH which in turn has acted on the adrenal cortex Neither riboflavin nor pyridoxine deficiency was associated with any regular changes in the adrenal cortex but both pantothenic acid and thiamine were There was one point about this work that bothered me a bit The actual number of rats in each deficiency group was 4 or 5 This may not affect the results but in view of the variation in the changes more animals should be examined before coming to any final conclusion As you know in pantothenic acid deficiency a decrease of ketosteroids associated with the loss of lipid depletion based on the staining reaction was also observed (8) A great many vitamins have not yet been tested for their effects on the function of the adrenal cortex

If I may I should like to go on to some of our own experiments about which I know more than I do about anyone else s We have been interested in the reaction of adrenalectomized as well as intact rats to deficiencies presumably affecting the adrenal cortex We were I might add stimulated originally to enter this field by Dr Fremont Smith We studied black and brown rats on diets

deficient in pantothenic acid (20) and got the same change in greying of the fur as Morgan reported We then went on to adrenalectomize these rats Our procedure, which perhaps may seem a little odd to you, was stimulated by the fact that we had started studying the effect of pantothenic acid deficiency in intact rats We therefore began with 30 to 35 day old rats The rats were placed on diets deficient in pantothenic acid at this age In the original experiments, the diets were only supplemented with riboflavin, nicotinic acid, pyridoxine, and vitamin A We did not supplement the first diet with biotin I don't know how much difference that made Now we carry out animals on a complete diet with adequate nutritional amounts of every known vitamin except pantothenic acid The rats were kept for 30 days on the diet The outstanding change, aside from the adrenal change was atrophy of the hair apparatus in the rat Microscopic sections showed that the hair apparatus was atrophied and little melanin remained in the hair follicles and bulbs When these rats were adrenalectomized, the atrophy of the hair apparatus which had occurred was reversed with extraordinary vehemence and the hair apparatus suddenly began to blossom as if there were a terrific stimulus to growth when the adrenal was removed (Fig 13) Butcher (4) observed this increase in fur growth in albino rats in 1939

Bauer Do you get the same response in the case of a sham operation?

Ralli The sham operation has absolutely no effect, not the slightest, and neither does shaving If you shave the pantothenic acid deficient rat and don t adrenalectomize it it does not usually regrow fur and if it does only a slight regrowth of a kind of fuzzy soft gray fur occurs When however you adrenalectomize such a rat and still leave it on a pantothenic acid deficient diet, the fur grows rapidly and is deeply pigmented Figure 11 is a picture of some of our adrenalectomized rats Some of these rats received calcium pantothenate postoperatively to maintain their survival When you feed calcium pantothenate to a previously deficient intact rat it begins to repigment its hair bulbs but the intact rat repigments in a spotty manner whereas the adrenalectomized rats develop a deep blue color to the skin all over the body The color is due to the fact that the overlying cutis is very thin and what you are seeing is reflection through the cutis of the concentrated melanin particles in the hair follicles and bulbs This as I said this morning can be arrested in these animals if DOCA is administered (21) As a matter of fact the local repigmentation that follows shaving in intact rats can be arrested by DOCA Pigmentation also occurs in

TABLE III*

ADRENALECTOMIZED RATS RECEIVING 1% NaCl FILTRATE FACTOR DEFICIENT DIET AND SUPPLEMENTS AS INDICATED

Experimental Conditions	No of Rats	Percent of Total Surviving — Days after Adrenalectomy								
		10	25	50	75	100	150	200	250	300
		%	%	%	%	%	%	%	%	%
Diet FFD +1% NaCl	105	50	2	0						
Diet FFD +1% NaCl +DOCA	57	75	13	9	0					
Diet FFD +1% NaCl +Cortin	53	70	15	4	0					
Diet FFD +1% NaCl +Ca pan	70	100	91	81	67	62	50	30	17	7
Diet FFD +2% NaCl	26	38	0							

ADRENALECTOMIZED RATS RECEIVING THE FILTRATE FACTOR DEFICIENT DIET AND SUPPLEMENTS AS INDICATED BUT WITHOUT NaCl
Percent of Total Surviving

Experimental Conditions	No of Rats	Days after Adrenalectomy				Average Survival in Days
		10	25	50	75	
		%	%	%	%	
Diet FFD No NaCl	26	7	0	0	0	6 2±2 5
Diet FFD +DOCA	17	50	0	0	0	11 5±5 0
Diet FFD +Cortin	18	0	0	0	0	5 3±0 5
Diet FFD +Ca pan	22	0	0	0	0	5 6±1 0
Diet FFD +DOCA +Ca pan	21	62	0	0	0	12 5±4 5

*Reprinted from *Endocrinology* 39 275 (1946)

the hypophysectomized black rats but it is not quite as intense nor is it as well distributed as in the adrenalectomized rats

During the course of the original studies (1940 1941) (20) we supplemented the diets of the rats after adrenalectomy with a rice bran concentrate This was before pantothenic acid had been identified as the factor in the filtrate fraction responsible for the graying of the fur and the adrenal changes We observed that the adrenalectomized rats survived for unusually long periods As soon as pantothenic acid was available this was used and we studied the effect of supplementing the diet after adrenalectomy with large amounts of calcium pantothenate (23) Table III gives the survival data on

FIGURE 13

FIG 13a NORMAL RATS on nu chow diet not adrenalectomized age 94 days The rats were shaved just prior to photographing The skin shows one type of arrangement of the normal pigment bands

FIG 13b *Rats 260 1 and 270 1* The diet had been deficient in the filtrate factors for 111 days at which time the animals were shaved and photographed The entire skin was pink and no pigment bands were found Age 147 days

FIG 13c *Rat A8 12* ADRENALECTOMIZED FILTRATE FACTOR DEFICIENT *10 days post* adrenalectomy and Rat *A8 12a* UNOPERATED FILTRATE FACTOR DEFICIENT Both rats were given the deficient diet when 35 days old On the 39th day of the diet *Rat A8 12* was shaved across the back and adrenalectomized The unoperated control rat was shaved across the back on the same day Ten days postadrenalectomy *Rat A8 12* died The area over the right shoulder was shaved at death This photograph was taken immediately after death *Rat A8 12a* was killed on the same day and the area over the right shoulder was shaved Note the diffuse bluish color of the skin of the adrenalectomized rat as compared to the pinkish color of the skin in the unoperated rat Total period of the deficient diet in both rats was 49 days Age at death both rats 84 days

FIG 13d *Rat A8 24* 17 days postadrenalectomy THIS RAT was on the diet deficient in the filtrate factors for 39 days prior to adrenalectomy After operation the rat was placed on the complete diet *Rat A8 24a* is the control. This animal was shaved across the lower back on the day that *A8 24* was adrenalectomized and was also changed to the complete diet on this day Seventeen days later both rats were shaved over the left shoulder and back killed and photographed In the adrenalectomized rat there is the characteristic bluish black color over the entire back and regrowth of glossy black fur over the area shaved at operation The control rat shows slight regrowth of fur over the shaved area and a return of the pigment bands

FIG 13e *Rat A11 13* 16 days postadrenalectomy complete diet for 18 days changed to diet deficient in filtrate factors after adrenalectomy *Rat A11 13a*, unoperated rat same age complete diet for 18 days then changed to diet deficient in filtrate factors *Rats A11 13* and *A11 13a* were begun on the complete diet when 32 days old After 18 days on the diet both rats were shaved over the entire back and *Rat A11 13* was adrenalectomized After adrenalectomy both rats were changed to the diet deficient in the filtrate factors This photograph was taken 16 days after shaving and adrenalectomy Note the regrowth of black fur in the operated rat and the fading of the pigment bands in the unoperated rat

FIG 13f *Rat A11 23* adrenalectomized rat 18 days postadrenalectomy complete diet for 36 days *Rat A11 23a* unoperated rat same age complete diet for 36 days *Rats A11 23* and *A11 23a* were begun on the complete diet when 32 days old After 18 days on the diet both rats were shaved over the entire back and *Rat A11 23* was adrenalectomized The rats were continued on the complete diet This photograph was taken 18 days after shaving and adrenalectomy Note the regrowth of thick black fur on the adrenalectomized rat and the moderate regrowth of fur in the unoperated rat The regrowth of fur in the unoperated rat is most marked in the areas of the original pigment bands

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1



Normal R ts

2



Filtrate Factor D fic ent

3



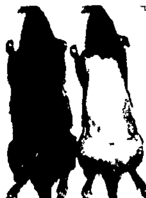
Adren l ctom z d Unoperated
A8 12 A8 12a

4



Adrenalectomi ed U pe ted
A8 24 A8 24

5



Ad e l ctom ed Unope ted
A11 13 A11 13

6



Adrenalectomized Unoperated
A11 23 A11 23a

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the hypophysectomized black rats but it is not quite as intense nor is it as well distributed as in the adrenalectomized rats

During the course of the original studies (1940 1941) (20) we supplemented the diets of the rats after adrenalectomy with a rice bran concentrate This was before pantothenic acid had been identified as the factor in the filtrate fraction responsible for the graying of the fur and the adrenal changes We observed that the adrenalectomized rats survived for unusually long periods As soon as pantothenic acid was available, this was used and we studied the effect of supplementing the diet after adrenalectomy with large amounts of calcium pantothenate (23) Table III gives the survival data on

FIGURE 13

FIG 13a NORMAL RATS on nu chow diet not adrenalectomized age 94 days The rats were shaved just prior to photographing The skin shows one type of arrangement of th normal pigment bands

FIG 13b *Rats 260 1 and 2 0 1* The diet had been deficient in the filtrate factors for 111 days at which time the animals were haved and photographed The entire skin was pink and no pigment bands were found Age 147 days

FIG 13c. *Rat A8 12* ADRENALECTOMIZED FILTRATE FACTOR DEFICIENT 10 days post adrenalectomy and *Rat A8 12a* UNOPERATED FILTRATE FACTOR DEFICIENT Both rats were given the deficient diet wh n 30 days old On the 39th day of the diet *Rat A8 12* was shaved across the back and adrenalectomized The unoperated control rat wa shaved across the back on the same day Ten days postadrenalectomy *Rat A8 12* died The area over the right shoulder was shaved at death This photograph was taken immediately after death *Rat A8 12a* was killed on the same day and the area over the right shoulder was shaved Note the diffuse bluish color of the skin of the adrenalectomized rat as compared to the pinkish color of the skin in the unoperated rat Total period of the deficient diet in both rats was 49 days Age at death both rats 84 days

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control group of rats. I should have mentioned that all rats, intact and adrenalectomized, received a solution of 1 percent sodium chloride in the drinking water.

Survival is calculated as the percent of the total number of rats adrenalectomized surviving after varying periods. Of the 105 rats on 1 percent NaCl and the deficient diet 50 percent survived for 10 days and 2 percent survived 25 days. None survived at the end of 50 days and the average survival was 11.9 days ± 5.1. When DOCA or adrenal cortical extract was injected 6 days a week in rats on the deficient diet the survival after 10 days was almost identical for the two groups. Seventy five percent of the DOCA treated rats and 70 percent of the rats receiving the cortical extract survived. In each of these groups a small number of rats survived 50 days but none survived for 75 days. When the diet was supplemented with calcium pantothenate survival was definitely increased. All of the rats were alive after 10 days and 50 percent survived 150 days. In order to check the completeness of adrenalectomy in the calcium pantothenate group salt was withdrawn from 20 rats at intervals during the course of the experiment. All but 2 of the rats succumbed within a matter of days and these 2 died but survived for long enough periods so that the completeness of adrenalectomy might be questioned. Salt withdrawal in all adrenalectomized rats was associated with a striking weight loss and a decrease in food consumption. In the group of rats that received a 2 percent solution of NaCl and the deficient diet after adrenalectomy the average survival was 9.4 days ± 5.0 and none of the rats survived for 25 days. I should like to mention that we found we were able to induce some pigmentation in intact rats if 2 percent sodium chloride was given to them regularly.

As can be seen in the lower part of Table III when NaCl was not given, and I should add that absolutely no NaCl was included in the diet, survival was sharply curtailed in all groups. Prolonged survival on the large doses of calcium pantothenate and NaCl seems to be the result of a combined effect.

We have also studied the critical requirement of pantothenic acid for the adrenalectomized rat (11). Remember these rats are only 60 days old. Table IV shows the effects of varying the pantothenic intake. When 0.03 mg of pantothenic acid was fed daily the mean survival was slightly longer than when the diet was deficient in pantothenic acid but survival was still strictly limited. This amount of pantothenic acid has been shown to be more than sufficient for optimal growth by intact rats 10 weeks of age (28). On 1 mg of pantothenic acid daily a larger percentage of the animals survived

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TABLE IV*

EFFECT OF VARYING DOSES OF PANTOTHENIC ACID ON SURVIVAL IN ADRENALECTOMIZED RATS

NaCl	Pan acid daily mg	No of rats	Percent of total surviving Days 10	25	50	75	100	150	Mean S D
1% in water	0	105	50	2	0	0	0	0	11 9±5 4
	0 03	19	74	0	0	0	0	0	14 2±4 3
	1 0	15	87	7	0	0	0	0	
	2 0	14	93	43	36	29	29†		
	3 0	31	74	16	16	16	16†		
	3 5	14	71	43	43†				
	4 0	22	96	77	73	50	41†		
	4–6	70	100	91	81	67	62	50	

† Experiments discontinued animals used for special studies

for 10 days When the dose of pantothenic acid was increased to 2 mg or more daily, there was a definite increase in percentage survival and in some of each group prolonged survival was obtained On 2, 3 and 3 5 mg daily less than half of the rats survived for 25 days or more When the daily dose of pantothenic acid was increased to 4 mg, 77% of the rats lived for 25 days or more

Analysis of the data on 105 control animals which were continued on the deficient diet following adrenalectomy showed that the weight of the rat at the time of adrenalectomy is one factor affecting its survival following the operation This data is shown in Figure 14 in which the weight of the rat is plotted against its survival after adrenalectomy The regression line was calculated by the method of least squares

Dr Dumm has worked out a survival index (Table V) as a method of expressing the survival of rats receiving various doses of pantothenic acid in a way which allowed for the variable of the weight at the time of adrenalectomy The survival index is the ratio of the median survival of rats on a given intake of pantothenic acid to the expected median survival of rats of the same weight ± 10 gm, on a diet deficient in pantothenic acid Table V shows the survival index for each group of adrenalectomized rats with reference to the dose of pantothenic acid received You see there is a sharp increase in survival index when we go up to 4 mg of pantothenic acid daily

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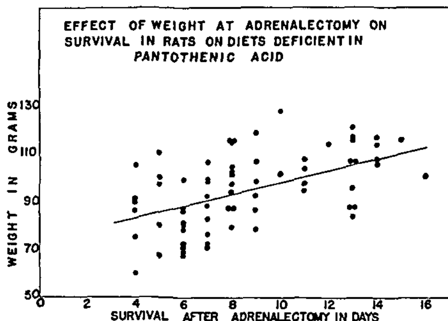


FIGURE 14 The weight in grams at the time of adrenalectomy is plotted against the survival after adrenalectomy in days. All rats had been maintained on a diet deficient in pantothenic acid for 30 days before adrenalectomy and w re continued on the same diet plus 1% NaCl after adrenalectomy

TABLE V*

THE SURVIVAL INDEX† OF ADRENALECTOMIZED RATS

Pantothenic acid mg /day	Number of rats	Mean weight at adr gms	Median survival after adr days	Survival * index
0 0	10	95	8	1 1
0 0	15	102	8	0 9
0 0	11	80	6	0 9
0 03	19	120	12	1 0
1 0	15	83	13	1 9
2 0	14	80	20	2 9
3 0	31	85	15	2 1
3 5	14	98	18	2 2
4 0	22	100	76	9 5
4–6	70	103	150	16 7

† The survival index is the ratio of the median urvival f rats on a given intake of pantothenic ac d to the expected survi al of rats of the same weight (±10 gm) on a diet d ficient in pantothenic acid

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We have now begun to test the other fractions of the vitamin B complex on survival Working still under the standard conditions of the preparation of the rat, we have added thiamine chloride to the diet postoperatively, a diet complete in every respect, and containing a minimal amount of pantothenate Adding thiamine chloride in an amount proportionately as great as was the increase in pantothenic acid, *we find that survival is not good It is almost* as if the excess of thiamine to the rats was imposing a burden on them The survival index of the rats on the thiamine chloride is 1 4 which puts them into the range of the deficient pantothenate diet The postoperative diet was adequate in every respect and the excess was in thiamine We are in the process now of investigating pyridoxine biotin folic acid and several other fractions *Furthermore we tested a diet which was adequate in all of the* fat soluble vitamins We added vitamin K and vitamin E which we had not added previously They had no effect on survival We withdrew choline and inositol from the diet at one time They don t seem to enter into the survival of adrenalectomized rats In the diet adequate in choline and inositol or in the diet without choline or inositol the survival figures were very much the same In addition to that we have tested the effect of the alcohol analogue of calcium pantothenate This should be a little more stable than calcium pantothenate However, we find that we do not get as good results on survival if we give amounts of the alcohol analogue in equivalent doses of pantothenic acid This may have something to do with its, utilization or excretion Animals and humans excrete more pantothenic acid when it is given in the form of the alcohol analogue than when it is given as calcium pantothenate Dr Rubin is of the opinion that the increased excretion is due to better utilization (24)

The adrenalectomized rat on salt and the large dose of pantothenate looks and behaves well The fur is in good shape As far as weight is concerned these rats gain at approximately the same rate as their intact controls for the first 30 days after adrenalectomy (Fig 15) The normal rat then continues to gain and the weight of the adrenalectomized rat usually levels off If salt is withdrawn from the adrenalectomized rat it loses very large amounts of weight rapidly—as much as 10 to 15 gm in 24 hours When salt is withdrawn from the intact control it will lose a small amount of weight for a day or so and then the weight levels off By this time the adrenalectomized rat has died

I have on certain occasions failed to remove both adrenals and found that under these circumstances pigmentation does not occur

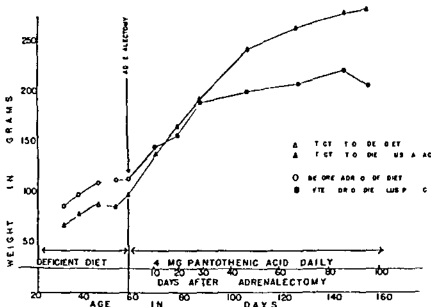


FIGURE 15* Compari on of growth curves in adrenalectomized and intact rats on 4 mg of pantothenic acid daily Average growth curve of 4 intact and 4 adrenalectomized male rats All rats were placed on a pant thenic acid defi ient diet when 32 days of age and were c ntinu d on the diet for ?6 days At thi time 4 of the rats were adrenalectomiz l and 4 were continued as intact controls an l each rat recei d 4 mg of pantothenic acid daily

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It is necessary to bilaterally adrenalectomize the animal to get the pigmentation Butcher (3) transplanted adrenals in black rats This results in atrophy of the medulla and as a result no pigmenta tion occurred because the adrenal cortex was still active

We have studied the excretion of both pantothenic acid and ascorb ic acid in adrenalectomized and intact rats on high doses of panto thenate and during the period of pantothenate deficiency (12) Results on the excretion of pantothenic acid are shown in Table VI The excretion of pantothenic acid is a trifle but not significantly higher in the adrenalectomized than in the intact rats of identical age The most marked differences occurred during the first week after pantothenic acid administration When the rats are placed on the deficient diet the excretion of pantothenic acid falls to very low levels Figure 16 gives the excretion of pantothenate by normal and adrenalectomized rats following the withdrawal of calcium pantothenate from the diet The adrenalectomized and intact rats had been maintained on a diet supplemented with 3 mg of panto thenate daily for 119 days before pantothenate was withdrawn from

TABLE VI*

THE EXCRETION OF PANTOTHENIC ACID BY ADRENALECTOMIZED AND INTACT RATS

Pantothenic acid intake 4 0 mg /day following a 30 day deficient period

Intact Rats				Adrenalectomized Rats		
Age days	Days on Pan Ac	Pan Ac excreted mg	% Pan acid excreted	Days after Adr & on Pan acid	Pan acid excreted mg	% Pan acid excreted
63	5	2 5	61	5	3 3	82
64	6	3 0	75	6	4 0	100
65	7	3 5	89	7	1 6	41
66	8	2 4	59	8	2 9	73
72	14	1 8	46	14	1 9	48
72	14	2 0	48	14	2 8	71
79	21	1 6	41	21	1 9	48
79	21	2 3	57	21	2 4	60
175†	115	1 9	41	115	2 3	62
Mean ± S D			60 ± 15			65 ± 20

Excretion of pantothenic acid after 20 30 days on the deficient diet was 0 002 to 0 005 mg per rat per day

† Rats had been receiving 3 mg pantothenic acid daily at this time

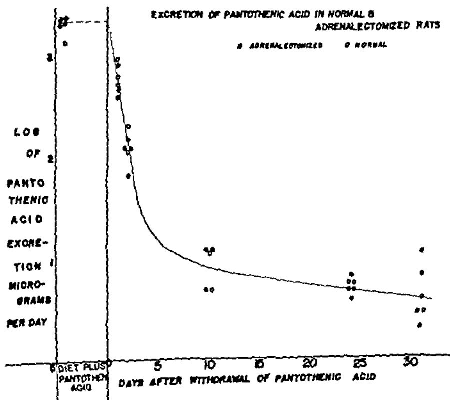


FIGURE 16*

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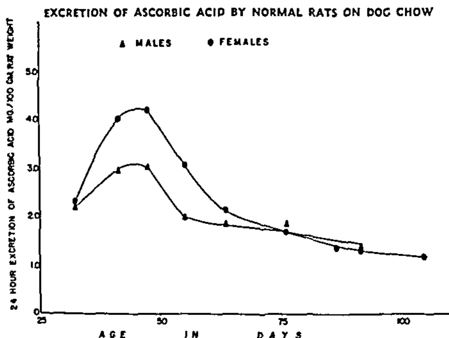


FIGURE 17*

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the diet Once the rat has survived adrenalectomy for a considerable period it will survive withdrawal of pantothenic acid, and under this circumstance its weight will drop more than does the weight of the control rat It seems as if, having reached adulthood even though adrenalectomized it does not require as much pantothenate after the first 50 days as it does initially after adrenalectomy This may be a question of storage by the tissues As regards the excretion of pantothenate after withdrawal you can see that the excretion decreases rapidly, but that there is no significant difference between the intact and the adrenalectomized rats

Because of the importance of ascorbic acid to the adrenal cortex we studied the excretion of this vitamin in intact and adrenalectomized rats on varying amounts of calcium pantothenate Figure 17 gives the excretion by normal male and female rat on the dog chow diet Females excreted more ascorbic acid than did males The hump in the excretion curve apparently is related to the maturation of the animal Similar changes have been reported for other animals (14)

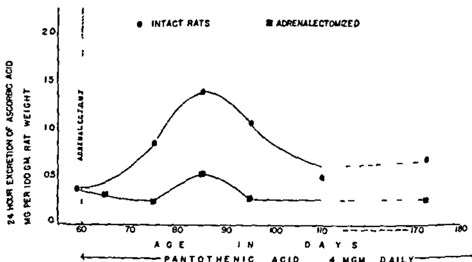


FIGURE 18

Bauer Does the increased ascorbic acid excretion take place on a constant intake?

Ralli No ascorbic acid was given Rats synthesize their own ascorbic acid Results shown are on the basis of 24 hour excretion in mg per 100 gm of body weight Figure 18 gives the excretion in intact and adrenalectomized rats on large doses of calcium pantothenate following a 30 day period of pantothenate acid deficiency Pantothenic acid deficiency is associated with a significant decrease in the excretion of ascorbic acid Please note that the ordinates in this figure represent half as much ascorbic acid excreted as the ordinates in the previous figure The addition of calcium pantothenate to the diet of the intact rat was associated with an increased excretion of ascorbic acid Again, the hump appears similar to the one appearing in intact rats at an earlier age This suggests that the deficiency of pantothenic acid interfered with the development of the animal, and when pantothenic acid was again added to the diet the delay in maturation was overcome You will note that there is a smaller hump at the same time in the excretion of ascorbic acid in the adrenalectomized rats We have data now on the excretion of ascorbic acid in hypophysectomized rats, and these animals excreted less ascorbic acid than any

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animals we have studied Dr Rubin informs us that the excretion of pantothenic acid can be affected by other fractions of the B complex, particularly biotin We are planning to enlarge the observations in the adrenalectomized animals to include studies on the excretion of the various components of the B complex Obviously, the low excretion of ascorbic acid that occurs in the adrenalectomized rat does not interfere with prolonged survival There were no obvious evidences of scurvy in any of the animals

To further investigate the state of these long surviving adrenalectomized rats receiving the large doses of pantothenate we subjected the animals to stress The forms of stress used were swimming in cold water and the injection of ACTH with which Dr Mote was kind enough to supply us

Loeb What dosage?

Ralli We gave 4 mg of ACTH per 100 gm per body weight This was injected into intact rats on diets adequate and deficient in pantothenic acid and the number of white blood cells and lymphocytes was counted at intervals of 2 hours for a period of 8 hours

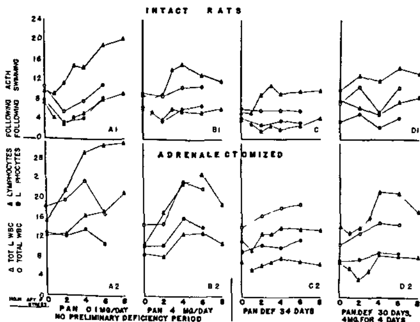


FIGURE 19

following the injections (13) Adrenalectomized rats were studied under precisely the same conditions The results on both groups are given in Figure 19 All points on these graphs represent from 6 to 12 experiments

At the top of the graph are the results on the intact rats The circles represent the results following swimming for 25 minutes in cold water at 25° C The triangles represent the offects of injecting ACTH The solid emblems indicate the lymphocytes and the clear emblems the total number of white blood cells Groups A and B were never subjected to any period of pantothenic acid deficiency, the diets were adequate in all nutritional substances but in the rats in the B group they were supplemented for 7 days before stress with 4 mg of calcium pantothenate daily The response of the intact rats in Group A1 is typical of what occurs in the normal rat, with a decrease in lymphocytes at the second hour following both forms of stress (10) The response of the rats in the B1 group was modified by the addition of excess calcium pantothenate to the diet and this is especially true of the total number of white blood cells In contrast to these 2 groups of rats C1 represents the results of stress in intact rats after a 30 day period of pantothenic acid deficiency As a result of the deficiency the total number of white blood cells and of lymphocytes was lower Neither form of stress provoked a response of any significance in the number of lymphocytes and only ACTH caused any change in the total number of white blood cells After 30 days of a diet deficient in pantothenic acid there is undoubtedly lipid depletion of the adrenal cortex Group D1 represents the results in rats given 4 mg of calcium pantothenate daily for 4 days after the 30 day period of deficiency The decrease in the number of lymphocytes occurred after ACTH but the maximum decrease was delayed until 4 hours after the injection Also after swimming there was a decrease in the number of lymphocytes after 4 hours

The lower part of this figure shows the results on the adrenalectomized rats who were treated in exactly the same manner as were the intact rats In these rats there were no adrenal glands to be stressed As a result of adrenalectomy the total number of white blood cells in the rats on the normal diet (A1) as well as the number of lymphocytes was increased Following the injection of ACTH no decrease occurred in the lymphocytes and at the 4th hour the number had risen significantly and continued to rise up to the 8th hour The total number of white blood cells rose to unusual heights

In the adrenalectomized rats in the B2 group the total number

of white blood cells rose after both swimming and ACTH, but had begun to decrease again by the end of the test The response of the lymphocytes after both forms of stress was similar No decrease occurred after 2 hours, the counts remaining the same as before the stress The number of lymphocytes then rose until the 4th or 6th hour, when they began to decrease The impression I get is that the addition of large amounts of pantothenate to the diet limits the exaggerated response which usually occurs following the injection of ACTH into adrenalectomized rats on normal diets

The adrenalectomized rats that were continued on the deficient diet after adrenalectomy (C2), and that had been on the deficient diet 30 days prior to adrenalectomy, showed very little response as regards the lymphocytes following either form of stress The total number of white blood cells after swimming however, rose moderately and there was a slight rise in the number of white cells after ACTH When previously deficient rats received calcium pantothenate for 4 days after adrenalectomy, ACTH provoked a greater response in the lymphocytes and the number of white blood cells but the lymphocytes after swimming still showed little response

Sayers May I interrupt at this point? Would you mind explaining that diagram the intact rats the second one from the top how were these animals treated?

Ralli The rats in this group (B1) were carried on the dog chow diet until they were 60 days of age At this time they were placed on the experimental diet, similar to the rats in the Group A1 but in addition they received 4 mg of calcium pantothenate daily They were kept on the high pantothenate diet for an average of 7 days and were then subjected to stress The adrenalectomized rats in Group B2 were treated similarly

Sayers You had a modified response in Group B1?

Ralli Yes The addition of an excess calcium pantothenate to a diet that was already adequate in pantothenate arrested the extent of the rise in the total number of white blood cells and to a less extent controlled the fall in the number of lymphocytes occurring 2 hours after stress or ACTH My impression of these findings is that the 'administered' stress seemed to exert less stress effect when the animal received an excess of pantothenate The rats that had been on the deficient diet for 30 days (C1) seemed to have no capacity to respond to stress and the adrenals were apparently exhausted When an excess of pantothenate was fed to such rats for 4 days (D1) a response to stress occurred The white blood cell and lymphocyte response to stress was also influenced by the dietary

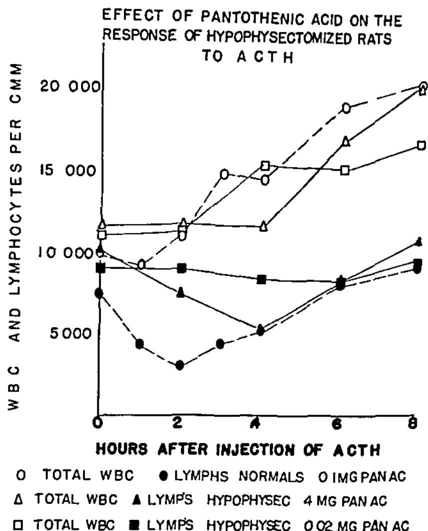


FIGURE 20

conditions in the adrenalectomized rats—even when the stress was ACTH

We have studied hypophysectomized rats under similar conditions We could not use exactly the same diets due to the capriciousness of the hypophysectomized rats appetites The diet was low in panto thenate, 02 mg *per 10 gm of food due to using small amounts of* Pard daily The high pantothenate diet had the same pantothenate content as that given the adrenalectomized rats Swimming was not attempted and the experiments were done with ACTH The results are shown in Figure 20 The response of normal rats is shown for com

pari on (circles) The results on the hypophysectomized rats on the low pantothenate diet are indicated by the squares ACTH provoked no change in the number of lymphocytes but there was some increase in the total number of white blood cells On the high pantothenate diet however, ACTH was followed by a decrease in the number of lymphocytes which was greate t after 4 hours The total number of white blood cells rose at the 6th hour, and was greater than in the rats on the low pantothenate intake We have found that the weight of the adrenals in the hypophysectomized rats was not influenced by the pantothenate content of the diet—the glands in both groups of rats were much smaller than in normal rats of the same weight from our colony (Adrenal of hypophysectomized rat on low pantothenate 4 8 ± 0 2 gm on high pantothenate 5 2 ± 0 4 gm normal rat 10 0 ± 0 4 gm)

Blood sugar determinations were done after the swim stress in intact and adrenalectomized rats on the deficient diets Table VII We have done blood sugar studies on diets containing 0 1 mg of pantothenate and on 4 mg of pantothenate The intact rats on this low pantothenate diet showed very little change in the blood sugar after swimming On the high pantothenate diet the blood sugar was lower at the start averaging 126 mg percent and it rose after the 25 minute swim to 134 mg percent The adrenalectomized rats both on the low diet and high diet, showed quite a different response The blood sugar fell in both cases but the adrenalectomized rat on the pantothenate deficient diet had a blood sugar before the swim of 107 mg percent with an S D ± 6, after swimming for 25 minutes the blood sugar had fallen to 69 mg percent

TABLE VII

INFLUENCE OF CALCIUM PANTOTHENATE ON BLOOD SUGAR OF INTACT AND ADRENALECTOMIZED RATS BEFORE AND AFTER SWIMMING

Condition of Rat	Mg % of Pantothenate Daily	No	Non Fasting Blood Sugar mg %		No	Fasting Blood Sugar mg %	
			Before Stress	After Stress		Before Stress	After Stress
Intact	0 1	10	13? ± 10	145 ± 4			
Intact	4 0	6	126 ± 7	134 ± 5			
Adrenalectomized Days							
4 – 13	0 1	12	107 ± 6	69 ± 4	9	61 ± 5	38 ± 4
4 13	4 0	14	119 ± 4	90 ± 4			

Blood sugar values are all expressed as mg % ± S E.

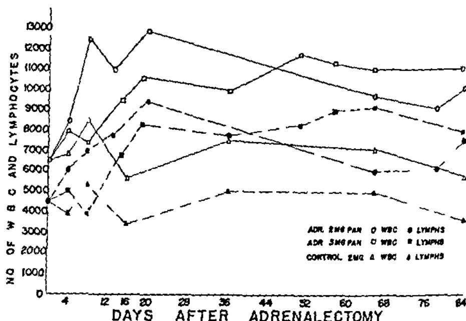


FIGURE 21 White blood cells and lymphocytes in adrenalectomized rats on large doses of pantothenic acid compared to the white blood cells and lymphocytes in intact rats on large doses of pantothenic acid

The rat on the high pantothenate diet, after being adrenalectomized started with a blood sugar of 119 mg percent which fell to 90 mg percent after swimming

Long Fed rats Dr *Ralli? They were fed when you started them* swimming?

Ralli We only have completed figures on one group of fasted adrenalectomized rats *The blood sugars were lower and fell to* very low levels *Our figures on the other groups are not completed*

We have only a few ascorbic acid determinations on the adrenals *after the 30 day period of pantothenate deficiency The range is* quite large due possibly to the handling of the animals at the time of adrenalectomy and I will have to report on this at some later *conference when we have more data*

Figure 21 illustrates the changes in the total number of white blood cells and lymphocytes in long surviving adrenalectomized rats on *a high pantothenate intake The intact rats are shown by the* triangles The adrenalectomized rats were on 2 mg (circles) and 3 mg (squares) of pantothenate daily Following adrenalectomy the usual increase *in the total number of white blood cells and* lymphocytes occurred As the period of the diet continued certain fluctuations were observed in the number of cells, but the number

was always greater than in the intact controls

As I said, this summary on the relation of vitamins to the functions of the adrenal cortex is not as thorough as I would like to have had it. It seems to me one may wonder whether or not there is such a thing as a specific nutritional requirement for the adrenal cortex as regards vitamins or whether a deficiency of vitamins may not be associated with a situation which stimulates the adrenal cortex exhausts it and so produces changes which follow a fairly definite pattern. Such changes would probably be lipid depletion atrophy, and, under stress hemorrhage. I would question I think the idea that any one vitamin specifically affects one zone of the adrenal cortex and I am not sure that it would not be better to discuss the relation of vitamins to the function of the pituitary and consider the adrenal as a part of the whole picture. Another point which deserves consideration is the effects of inanition on the adrenal cortex. There have not been too many studies done on this aspect of the problem. Bessey (2) did some studies in order to compare the effects of starvation to the effects of a scorbutic diet in guinea pigs and found that starvation did not cause changes in the adrenals similar to what he observed in scurvy. D Angelo and his associates (7) reported that starvation in guinea pigs caused an increase in adrenal size which was roughly proportional to the degree of body weight loss and to the duration of the inanition. They found that the adrenal hypertrophy involved primarily the fasiculate and to a lesser extent the reticularis zones. The zona glomerulosa was atrophied. These findings suggest that inanition also may constitute a form of stress to the adrenal

All cells require certain metabolites if they are to function normally. It is not unreasonable to think that the cells of the adrenal cortex, which are particularly important to the general metabolic state of the body and are being continually called upon to secrete their hormones require more of certain substances than cells that are less active physiologically. Since it is important to the general physiology of the body to maintain the integrity of the adrenal cortical cells it may be that a deficiency of any cellular component will render the cortical cells more susceptible to stress

It was of considerable interest to us that the reaction of the adrenalectomized rats to stress was influenced by the pantothenate content of the diet. This was true even when the stress was ACTH a hormone presumably specific for the adrenal cortex. This raises the interesting possibility that a trophic hormone may elicit a response even if its specific target gland is absent provided a propitious situation exists in the tissues

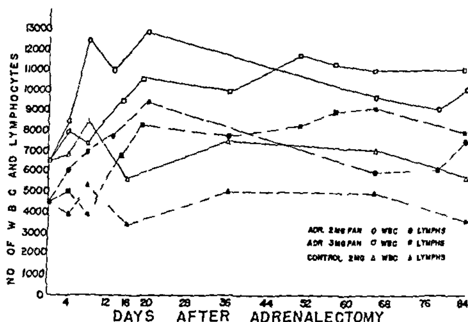


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DISCUSSION

Conn Dr Ralli you said something about large amounts of sodium chloride affecting the pigmentation of normal animals Would you enlarge on that a little?

Ralli Dr Spoor (Spoor H J and Ralli E P Chemical studies in melanogenesis in normal and adrenalectomized rats *Endocrinology 35, 325 (1944)*) who worked with us reported the chemical analyses of the hides of the rats for precursors of melanin and preformed melanin Among the rats studied were intact rats on 1% NaCl, 2% NaCl and animals deprived of water He found that dehydration by means of 2% NaCl produced a higher melanin content (17 8 mg /gm tissue) than was found in normal dehydrated rats (13 8 mg /gm tissue) or than was found in intact rats (14 6 mg /gm tissue) The concentration of melanin was not as great as in the adrenalectomized rats (23 4 mg /gm tissue)

Conn You increase the pigmentation?

Ralli It seemed that way

Conn Desoxycorticosterone on the other hand decreased the pigmentation in the adrenalectomized animal is that correct?

Ralli That is correct I would like to point out however, that to obtain inhibition of pigmentation DOCA should be started within 24 hours after adrenalectomy I should also like to point out that if you delay the administration of large amounts of pantothenic acid to the adrenalectomized rat survival is curtailed In other words the therapeutic effect of pantothenic acid on survival depends to some extent on the time after adrenalectomy when its administration is begun The effect on survival occurs if we delay administration for as long as 24 hours so there must be some situation in the animal that is being altered that has to be taken hold of rapidly

Sayers What is the requirement of the normal intact rat for pantothenic acid?

Ralli About 03 mg per day This depends on the age of the

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ascorbic acid under certain circumstances There are a number of other factors besides adrenocorticotrophic hormone which influence the concentration of ascorbic acid in the adrenal and also a number of factors besides the adrenal cortex which influence the number of circulating lymphocytes There are two points which appear quite apparent to me in these experiments First the number of circulating lymphocytes in the pantothenic acid-deficient animal is initially low

Ralli That is right

Sayers The possibility exists that you might get less of a decrease under these circumstances

Second the pantothenic acid-deficient animals have been under stress for a long period of time before you subject them to the swim stress You are applying the acute stress to animals subjected to chronic stress and I would like to know what effect the chronic stress had on the response as measured by the number of circulating lymphocytes Lymphocytes of stressed animals may not respond as well as those of normal animals I am just bringing these up as possibilities of interpretation here I struggled with the same points regarding ascorbic acid There are a number of possible interpretations other than the one you reach i e, that it is a decrease in the functional activity of the adrenal cortex in pantothenic acid deficient animals

Ralli It is hard to be sure of an absolute explanation on the points you have brought up and it is true that after a 30-day period of pantothenate deficiency the rats have probably been subjected to a form of stress Therefore the lack of response of the intact rats to ACTH or swimming may well reflect exhaustion of the adrenals The point which interested me particularly was the effect of ACTH on the adrenalectomized rat and I can assure you that these were all completely adrenalectomized as we tested each rat several days after the experiment by withdrawing salt from their diets and all the rats died Apparently even in the absence of the adrenal a response to the injection of ACTH is manifested and this response can be conditioned by the pantothenate intake Furthermore in both the intact and adrenalectomized rat a short period of pantothenate administration (4 days) was associated with changes in the lymphocyte and leucocyte response In the rats in which the adrenals were absent (Group D2) this suggested to us that pantothenic acid was producing some situation in the tissues on which ACTH was acting

Long Dr Ralli if you had a chronic stress for 30 days due to another vitamin deficiency would that be a better control?

Ralli We are gradually going through all of the fractions of the

rat The older the rat, the less the requirement There is a range but most observers report that 0.3 mg per day is adequate

Fremont Smith Is there pantothenic acid in the adrenal cortex? Has anybody tested for that?

Long Yes it is one of the richest sources

Bloch After liver

Ralli I would like to add another point Dr Dumm, working with me has done acetylation studies in intact and adrenalectomized rats on diets deficient and high in pantothenic acid PABA is injected intraperitoneally and the amount acetylated determined There was no difference in the amount acetylated by intact and adrenalectomized rats on diets deficient or high in pantothenate However, in both groups, when the diet was deficient acetylation was low, 58 ± 4% for the intact, and 61 ± 4% for the adrenalectomized rats Similarly when the diet was supplemented with 4 mg of pantothenate daily the percent acetylated increased equally in both groups of rats The studies were carried on over a total period of 113 days and we were interested in observing that after 90 days on pantothenate the percent acetylated by both the intact and adrenalectomized rats rose to 80% This high percent acetylated is unusual and probably reflects the tremendous amounts of pantothenate in the diet

Sayers Dr Ralli you did not give an interpretation of the experiments the swim stress experiments and change in lymphocytes

Ralli I should be interested in hearing your views on this as I thought we had given ours

Sayers I am speaking of the data published in the Proceedings of the Society for Experimental Biology and Medicine in which you demonstrated that animals on pantothenic acid deficient diet had a poor response to swimming in the sense that there was less of a decrease in circulating lymphocytes when exposed to swim stress Does this mean that the adrenal cortex is less active secreting less hormone because of pantothenic acid deficiency or are there certain other variables which are influencing the number of circulating lymphocytes I wonder if you would expand on that

Ralli I think it would be our feeling that the decrease in the lymphocytes reflected changes in the adrenal cortex However it is interesting that the response of adrenalectomized rats as regards circulating lymphocytes was influenced by the dietary treatment of the animal and probably by the pantothenate content of the diet

Sayers There are a number of points that come up in this connection One is the question of indices measurement of adrenal cortical activity I have gotten into difficulties myself regarding

We have studied animals under situations of acute fast (complete withdrawal of food but given water) We have also studied chronic manition tryptophane deficiency and low protein intake To make a long story short we have come to the conclusion that the pituitary adrenal system seems to have a high priority on protein building blocks for the synthesis of adrenocorticotrophic hormone The adrenal cortex itself seems to have a high priority on substances necessary for the production of its own hormone Under these various circumstances of dietary deficiency and stress we find that the content of ACTH in the pituitary is a little less than normal but not significantly so Under these same circumstances the supply of gonadotrophic hormone is indeed poor In spite of the fact that the animal is in this malnourished state the adrenal cortex will hypertrophy and there will be marked depletion of cholesterol and ascorbic acid in response to stress

Quite recently we have been able to get adenohypophyseal tissue from a human subject who was very cachexic at the time of death There had been carcinoma involving the gastrointestinal tract The pituitary had a very high concentration of ACTH probably just as high as we found in subjects who died in a well nourished state We have reached the tentative conclusion that the pituitary adrenal system is quite different from the pituitary gonad system It seems to have a fairly high priority on essential building blocks

White May I ask some questions Dr Sayers in connection with your discussion? You mean that in circumstances of restricted food nutrients both as to quality and quantity the pituitary continues to elaborate ACTH and retains its capacity to discharge and to resynthesize the hormone?

Sayers That is correct

White Have you studied the fasted castrated animal by chance? Dr Szego has studied in our laboratory the castrated male animal with respect to its capacity to respond to stress such as fasting In such an animal in which it is known there is an overproduction of gonadotrophin the response to fasting as measured by things which ACTH would do if it were released is very much less than it is in the normal fasted animal We concluded tentatively that in an animal where the pituitary is so actively concerned with making gonadotrophins there may be less capacity or time or effort or materials for making ACTH

Sayers That is a very interesting type of experiment We were thinking of the same sort of story in regard to the adrenalectomized animal That animal is so busy making adrenocorticotrophic hormone he may be having difficulty in regard to gonadotrophic hormone

B complex At the present time, we are testing the administration of large amounts of thiamine We also thought we would test vitamin C, but of course there is the difficulty of producing vitamin C deficiency, and we are thinking of using the antimetabolite

Long Is this gluco ascorbic acid?

Ralli Yes

Long You would have to be very careful whether you produced scurvy or something else

White I was just going to say, how do you control the factor of chronic inanition as a stress?

Ralli At the end of the 30 day deficiency period the rats are not normal but they are not, as a rule, in poor condition

White But their food intake is less than normal?

Ralli The rats eat approximately 6 to 8 gm of diet daily as compared with 10 to 12 gm in the normal As to the growth curves, during the first 2 weeks of the deficient diet, they continue to gain weight, and then begin to level off

White The thing that comes to mind is the difficulty of controls in the sense that restriction of the calories is a potent activator of the pituitary adrenal cortical mechanism

Ralli What you say is probably true, but we felt that administering the calcium pantothenate for a short period would give us some clue and as you saw the response to ACTH and swimming was different after 4 days of pantothenate Furthermore in the adrenalectomized rat there is no adrenal gland present to be disturbed I wondered in the intact rat if any of you felt that these deficiencies might have a direct effect on the anterior pituitary? Is there any reason to think that pantothenic acid deficiency operates solely through the adrenal?

Long None whatsoever You can have a sick pituitary perhaps even faster than an adrenal I think that this has been well established in relation to the gonadotrophic function, where the estrus cycle will cease in rats on a deficient diet, but the ovary will still respond when gonadotrophin is given This has been reported for at least half a dozen vitamin and amino acid deficiencies

Sayers We have been very much interested in the problem of the effect of diet on the functional capacity of the pituitary adrenal system We have approached it as follows we have determined the content of ACTH in the pituitary We have measured the ability of the animal to discharge ACTH from the anterior pituitary after stress by measuring the concentration of adrenal cholesterol and ascorbic acid In other words we have measures of both the content of ACTH in the anterior pituitary and the rate of discharge of ACTH

age factor but to different degrees of pantothenic acid deficiency

Ralli I don t think I could answer that question It would depend on the age of your rats how much they were eating what diet you were using etc Where our diets were concerned, Dr Rubin was kind enough to determine the pantothenic acid content in all of our diets after they were mixed We did this to be sure that the pantothenate was well distributed throughout the diet and we sent samples of the diets to him for analysis

Gellhorn One last point if I may You mention that the adrenalectomized animals under the test of swimming showed the fall in blood sugar both on the lower and high pantothenic acid content I would suggest that this is due to the vagal insulin discharge and if you would cut the vagi below the diaphragm you would no longer get this

Ralli This may be true, we found the average blood sugar of the adrenalectomized rat on 4 mg of pantothenate before swimming was 119 mg % After swimming the blood sugar fell to 90 mg % In the intact rat on the high pantothenate the blood sugar before the swim was 126 mg % and this rose to 134 mg % The intact rat on the low pantothenate diet, before swimming was 132 mg % and after swimming was 145 mg %

Long It is important to know the time relationship between the exercise and the last meal because of the liver glycogen content at the time you start to swim You have to have some standardization if you are using fed animals If the animal has three or four percent glycogen in the liver the rate of fall of blood sugar will be much slower than if the animal has practically no glycogen at the time it exercises That is why I asked you before about fasted animal It seemed to me that their use would make the differences much sharper and clearer and would not be complicated by varying content of liver glycogen

Ralli I regret that we have not more data on the fasted rats

Ingle Dr Ralli have you done studies of the survival of adrenalectomized rats on saline which had not been depleted with pantothenic acid?

Ralli Yes if we adrenalectomize young rats which have been fed diets high in pantothenate prior to adrenalectomy survival after adrenalectomy will be prolonged even if the animal is placed on a deficient diet after it is adrenalectomized The amount of pantothenic acid fed the rat before adrenalectomy will also influence survival Furthermore after rats on a high pantothenate intake have survived adrenalectomy for a fairly long period you can stop the pantothenate and they will survive reasonably well for several

production That has a great number of implications, but we have not gone far enough to make definite conclusions

Long What happens if you take out both the gonads and the adrenals?

Fremont Smith You should test the priorities there because you might have the life saving adrenotrophic taking priority It would be an interesting experiment

Gellhorn Did I understand correctly that on your normal rats, fed a small amount of pantothenic acid the blood sugar rises were missing and that there was a hypoglycemia in the animals which were receiving normal amounts of pantothenic acid?

Ralli Following stress in the intact rats on the high pantothenate diet there was an increase in the blood sugar, but in the adrenalectomized rats the blood sugar fell, but not to hypoglycemic levels On the low pantothenate diet in the intact rats, an increase also occurred in the blood sugar following swimming

Gellhorn I have been puzzled in recent experiments that rats under conditions of stress do not show a marked hyperglycemia as has been observed by many authors including ourselves I wonder whether there is perhaps a pantothenic acid deficiency in our diet We just give ordinary dog chow You mentioned that this is very low

Ralli Yes there is only 0 1 mg of pantothenate in 15 gm of the dog pellets

Gellhorn That is inadequate Secondly I would like to know is there any evidence that the sensitivity to pantothenic acid is a function of age?

Ralli I can only say this, that the older rats will not show the obvious changes of pantothenic acid deficiency that you will get in the growing rat If you want to get the characteristic lesion of changes in the fur you have to work on the younger rat The pantothenic acid requirement of the rat decreases with age

Gellhorn I was asking the question for this reason a few years ago we studied the effects of certain stresses such as anoxia on rats of different ages and we found that the young rats responded with relatively marked hyperglycemia As the animals grew older the rise in blood sugar became less and in part it was reversed at the time, I interpreted these changes as due to a change in the balance between the sympatheticoadrenal and vagal insulin system (Safford, H and Gellhorn, E *Proc Soc Exper Biol & Med* 60 247 *(1945)*) *It is of course* possible that if pantothenic acid, about which I did not know anything at that time is a factor determining the degree of blood sugar rise the results could be due not to any

If the adrenalectomized rats on a lower pantothenate intake such as 2 mg daily, survive for 30 days, they will continue to survive for fairly long periods We still have to try variations of pantothenate administration such as the minimal dose required after the rats have survived 30 days on the large doses of pantothenate I should imagine that as the rat gets older, even the adrenalectomized rat, less pantothenate might be needed The growing rat is really under some stress from the pituitary and perhaps during that time is in a situation in which pantothenic acid is relieving or helping to balance this stress

Long Are there any other questions?

White Do young rats on a pantothenic acid deficient diet show histological changes of the kidney?

Ralli No—not unless the rats are kept on it for very long periods of time, and even then the changes are irregular and do not seem to be advanced After 30 days on the deficient diet no pathological lesions are encountered in the kidney

weeks This suggests some storage of pantothenate, although I was under the impression from the literature that the body does not usually store pantothenic acid in large amounts

Bloch After you withdrew pantothenic acid for 30 days there was appreciable excretion I think it amounts to 30 mg over 30 days There must be very appreciable capacity for storage

Ralli Do you know of any work which shows storage of pantothenic acid? We have not done tissue analyses On the basis of excretion, when pantothenic acid is removed from the diet, the excretion falls very rapidly, and then continues at a low level The continued excretion in the absence of any pantothenate in the diet may be associated with the loss of body weight

Long How did you determine the pantothenic acid in the urine? Was it by microbiolological methods?

Ralli In determining the pantothenic acid excretion, we have the cooperation of Dr Rubin of the Hoffmann LaRoche Company He uses the microbiological method We are also trying to study the role of pantothenic acid in human nutrition and have fed patients with various metabolic diseases very large amounts of calcium pantothenate Excretion studies on these patients have given us results similar to what we find in the rats

Ingle Do you conclude from your studies on the rat that removal of the adrenals increases the optimal requirement for pantothenic acid?

Ralli I would say this, Dr Ingle, that the amounts of pantothenate necessary to produce prolonged survival are so large that I do not think they have anything to do with the requirements in the ordinary nutritional sense The impression I get is that we are surfeiting some system in the body with this metabolite We had hoped that the acetylation studies would give us a clue as to the mechanism of the action of pantothenate However the results in the normals and in the adrenalectomized rats were identical In this respect it may be that when only 50% acetylation occurs that you have a critical situation which is too critical for prolonged survival in the adrenalectomized rats

Ingle Using young adult rats force fed a synthetic diet which has about one tenth of a milligram per day of pantothenic acid and giving them saline to drink will maintain their lives indefinitely and I think in the last ten years I have not lost more than four or five animals on that regimen Animals of that age are very much easier to maintain on salt than the immature animals which you are using

Ralli I think confirmation of that is also present in our results